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Winter Distribution of Ringed Seals (*Phoca hispida*) in the Barrow Strait Area, Northwest Territories, Determined by Underwater Vocalizations

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Calvert, W., and I. Stirling. 1985. Winter distribution of ringed seals (*Phoca hispida*) in the Barrow Strait area, Northwest Territories, determined by underwater vocalizations. Can. J. Fish. Aquat. Sci. 42: 1238–1243.

In order to assess underice distribution of ringed seals (*Phoca hispida*) in winter, we made recordings from 23 to 30 April 1982 at 32 sites chosen to represent different habitats in the High Arctic. By regressing the vocalization rate at each site against variables for habitat quality, we found that sites in smooth interisland channels had significantly more vocalizations than sites in bays, and sites with frequent human activity had vocalization rates similar to the overall average. Although differences in vocalization rates correlated with some measured and estimated habitat variables, there was too much overlap between sites for vocalization rate alone to be useful in separating suitable and unsuitable pupping habitat. Recordings made at one site over 4 d showed a diel cycle in which vocalization rate was highest from about 08:30 to 16:30 and lowest at night.

Dans le but d'évaluer la répartition du phoque annelé (*Phoca hispida*) sous la glace en hiver, on a effectué des enregistrements du 23 au 30 avril 1982 à 32 emplacements choisis comme représentant différents habitats du haut Arctique. En effectuant une régression de la fréquence de vocalisation à chaque emplacement en fonction des variables de qualité de l'habitat, on a découvert que les emplacements situés dans les chenaux entre les îles faisaient l'objet de plus de vocalisations que ceux qui étaient situés dans des baies; par contre, les emplacements souvent fréquentés par l'homme avaient des taux semblables à la moyenne globale. Les différences entre les taux de vocalisation étaient en corrélation avec certaines variables relatives à l'habitat quantifiées et calculées, mais il y avait trop de chevauchement entre les emplacements pour que seule la fréquence de vocalisation soit utile pour différencier les habitats convenant ou non à la mise bas. Des enregistrements effectués à un emplacement pendant 4 jours ont révélé un cycle nycthéméral : la fréquence de vocalisation était plus élevée de 8 h 30 à 16 h 30 environ et plus basse la nuit.

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Ringed seals (*Phoca hispida*) are the most abundant and ubiquitous marine mammals in the High Arctic. They are year-round residents, capable of maintaining breathing holes in the area of their choice after freeze-up. Preferred wintering habitat is stable annual ice that is over moderate water depths and has sufficient pressure ridges to collect snow drifts (McLaren 1958; Smith and Stirling 1975).

Adult ringed seals appear to establish territories at freeze-up and remain there until breakup (Smith and Hammill 1981). Where drifts are deep enough, they excavate subnivean lairs for haul-out sites above their holes. Pregnant females give birth in late March to early April, and the lairs provide the pups with some protection from predators and cold. Areas suitable for birth lairs are apparently localized and their distribution can vary greatly between years (Smith and Stirling 1978; Smith et al. 1978).

In a preliminary study of ringed seal habitat in northern Peel Sound and western Parry Channel (Viscount Melville Sound and Barrow Strait) during April and May 1975 and May 1976, Smith et al. (1978) found that western Barrow Strait and northern Peel Sound had the highest densities of birth lairs. During aerial surveys conducted in late June and early July 1980 and 1981, the highest densities of ringed seals in the High Arctic were recorded in Barrow Strait and Wellington Channel (Kingsley et al. 1985). The aerial surveys were later than the birth lair surveys because they were timed to coincide with peak haul-out, but before breakup, to obtain an index of abundance and distribution which was assumed to represent winter conditions. This assumption was somewhat tenuous, since the extent of stable annual ice and time of breakup can vary greatly between years and areas, and some seasonal movements may have already occurred. Regardless, it is clear that Barrow Strait is one

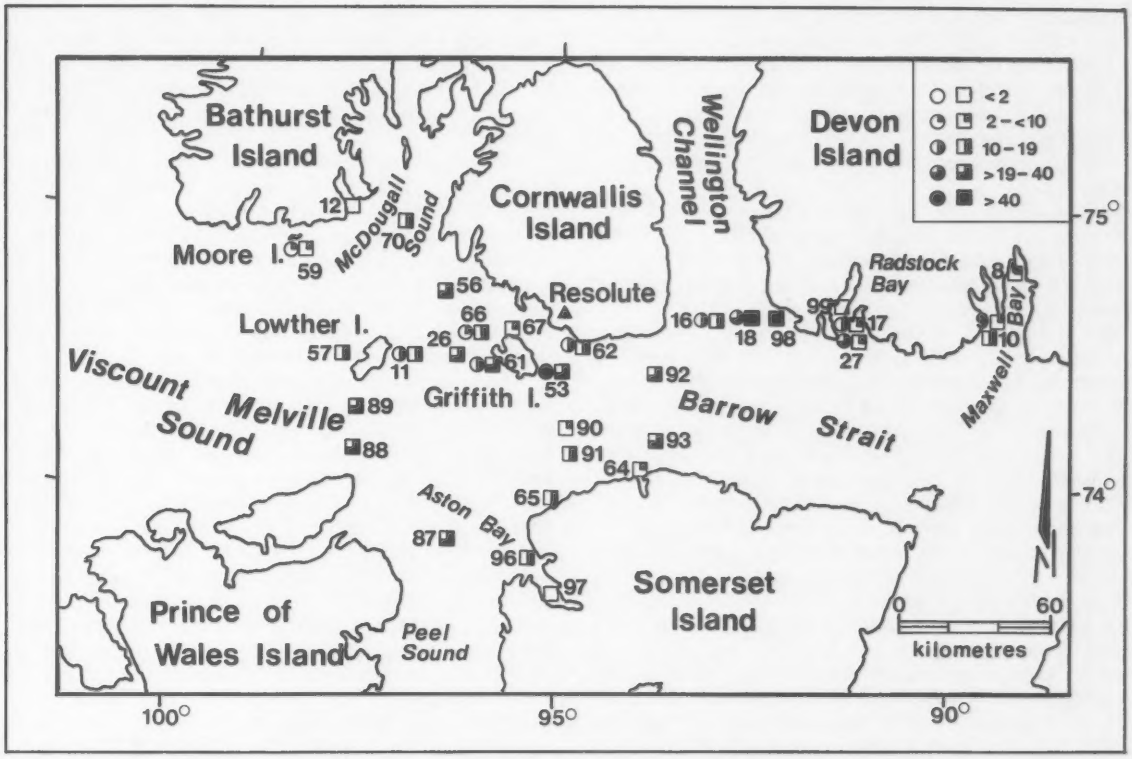

FIG. 1. Ringed seal vocalization rates at sites in the study area. Circles: 26–28 April 1981; squares: 23–30 April 1982.

of the most important areas in the High Arctic for winter residence and breeding of ringed seals.

Many of the proposals for industrial development in the Arctic include marine components such as offshore exploration for hydrocarbons, tankers for shipping ore, liquefied natural gas, or oil to southern ports, or supply ships for settlements (Stirling and Calvert 1983). Independent estimates suggest that there may be 900–1000 passages of ships in arctic waters by the turn of the century (Canadian Arctic Resources Committee 1980). Some proposals, such as that of the Arctic Pilot Project, include the use of large specially built ice-breaking ships which would operate year-round in Parry Channel.

Areas in Parry Channel important for ringed seal wintering, and especially pupping, need to be defined in order that they receive consideration during the planning of shipping lanes. In winter, few seals haul out on the snow surface; thus, aerial surveys are not possible. Late winter (March–May) birth lair surveys with a dog (Smith and Stirling 1975, 1978) are probably the best way to evaluate ringed seal breeding habitat, but as outlined in Stirling et al. (1983), they are labor-intensive and dependent on trained dogs and handlers.

Smith and Stirling (1975, 1978) found a relationship between vocalization rates and the number of ringed seal birth lairs found in Amundsen Gulf, and Stirling et al. (1983) reported some relationships between underwater vocalization rates and habitat type in the High Arctic. The vocalization rate increases in late April and probably remains high through May, possibly in association with increased reproductive activity (Stirling et al. 1983). However, vocalizing is probably necessary for the maintenance of a social structure around breathing holes in the fast ice and therefore takes place to some extent at all times during winter in all age and sex classes. If so, vocalization rates might be useful for assessing underice distribution and relative abundance of ringed seals in winter. Our objective was to determine the winter distribution of ringed seals in the Barrow Strait area in late winter 1982 through the analysis of underwater vocalizations. We hypothesized that because the movement of ringed seals after freeze-up is limited by the distribution of their breathing holes, they would be most abundant during the winter in areas where the best birth lair habitat would be in the spring.

Methods

We surveyed 23–30 April 1982 to precede breakup and coincide with the time of maximum vocalization rate. We chose 32 sites in Barrow Strait and adjacent areas (Fig. 1), of which 21 had been sampled and numbered in previous surveys (Stirling et al. 1983). The sites represented a variety of habitats, and were chosen to test hypotheses generated from previous studies of ringed seals and arctic habitats (Stirling et al. 1981, 1983; I. Stirling, unpubl. data) and from our observations of polar bear (*Ursus maritimus*) distribution and hunting behavior (Stirling and Latour 1978; Stirling et al. 1978; I. Stirling, unpubl. data).

Site 87 was selected because Smith et al. (1978) reported a high density of birth lairs in northern Peel Sound. The ice at site 57 is rough and tightly compacted against Lowther Island by the easterly flow of currents from Viscount Melville Sound, and Smith et al. (1978) reported low densities of birth lairs there.

Sites 59 and 70 are in smooth ice areas over fairly shallow water where bearded seals (*Erignathus barbatus*) occur, and we predicted lower densities of ringed seals there. Two sites (16 and 18) are in southern Wellington Channel because high densities of ringed seals have been reported there (Kingsley et al. 1985) although, subjectively, the apparent suitability of the area for birth lairs has appeared to us to be highly variable over the past few years.

We also hypothesized that human activity might deter seals from using an area, resulting in lower vocalization rates. Local Inuit hunt near sites 53, 62, and 67. Site 62 receives more disturbance from oversnow machines and low-level aircraft than anywhere else in Barrow Strait.

Sites in bays were chosen to test the hypothesis that their smooth ice, with few pressure ridges and snow drifts, would be poor birth lair habitat and therefore have few seals (Stirling et al. 1981). Sites in rough ice, which forms along coastlines, in the mouths of bays, and around the islands in northern Barrow Strait, were chosen to test the hypothesis that since they consistently appear to be more suitable for birth lair habitat, more seals would winter there than in the smoother and less drifted areas further offshore in the interisland channels. We classified each site for ice habitat as being in a bay, in coastal rough ice, or in an interisland channel.

Sites were reached with a Bell 206B Jet Ranger helicopter. After landing, the engine idled for 2 min to allow sufficient cooling before shutting off. A hole for the hydrophone was drilled through the ice with a 10-cm hand auger. To reduce the possible effects of a diel cycle in vocalization rate, all recordings on the helicopter survey were made between 08:00 and 18:00 C.S.T. Recording techniques and equipment followed Stirling et al. (1983), except that we extended recording time from 10 to 20 min at each site to test for a short-term response to noise disturbance. Recording usually began within 15 min of landing. To determine if the noise of the helicopter and of drilling a hole caused the seals to vocalize less for a short time, we divided each 20-min recording into four 5-min segments for analysis. We timed recordings with a stopwatch in the field and again during analysis in the laboratory.

Because ringed seal vocalizations are often faint and easily masked by ambient noise, especially wind (Stirling et al. 1983), we usually sampled when wind speeds were below 10 km/h. Also, we recorded in bays or near shore as near as possible to the time of slack tide to reduce noise from cracking or grinding ice. When the data were tabulated, a subjective estimate was made of the degree of noise from wind and ice. The background noise from tape hiss was also evaluated on the same scale.

After recording, we took 10 measurements of snow depths at 10-m intervals on each of three headings (0°, 120°, and 240°) from the recording site and averaged the 30 values. Each site was also rated on an ascending subjective scale from 1 to 5 for suitability as birth lair habitat, based on Smith and Stirling (1975): 1, flat ice with little or no snow cover; 2, occasional pressure ridges with small drifts (<50 cm deep) and little or no snow between ridges; 3, pressure ridges with medium drifts (<1 m deep) and most ice between ridges snow-covered; 4 and 5, increasing numbers of old, stable pressure ridges and ice hummocks with deeper (1–1.5 m) and longer (5–10 m) drifts.

To test for a diel cycle, we established a camp on the ice at site 61, where we had heard a high vocalization rate at 10:30 on 23 April and suitable birth-lair habitat had been found in previous years (Smith et al. 1978). Recordings were controlled by an interval timer so that the tape recorder came on automatically for 20 min ever 2 h from 20:30 on 23 April to 16:30 on 27 April. There were some equipment problems, resulting in two recordings of 9 and 15 min, while four others were too poor for analysis. The recordings used were timed to the nearest 0.01 min and analyzed as calls per minute, extrapolated to vocalizations in 20 min.

We analyzed each recording using the lexicon given in Stirling et al. (1983). Because we cannot identify age and sex class from vocalizations, nor know if all age and sex classes use the same vocalizations and vocalize at the same rate, all types of vocalizations were weighted equally for this analysis, and vocalization rate is the total of all vocalizations in the 20-min recording, expressed as calls per minute. In the comparisons between sites, we assumed that vocalization rates reflected relative abundances of seals beneath the ice.

We used stepwise forward multiple regressions to investigate winter habitat selection. Critical levels were 0.01 to enter and 0.05 to leave. Initially, the dependent variable was the square root of the total vocalizations in each 5-min segment, the sampling unit was site, and the independent variables were snow depth and sets of binary variables (Draper and Smith 1981) for segment number (4 variables), time (24 variables), ambient noise (4 variables for each type), ice habitat class (3 variables), and birth lair index (5 variables). After segment number was not chosen by the regressions, we used the square root of the total vocalizations in each 20-min recording as the dependent variable and eliminated segment number from the regressions. The regressions were unweighted for variation in vocalization rate.

For the recordings made every 2 h for 4 d at site 61, we regressed the square root of the vocalizations in 20 min against a family of 12 binary variables (from "time >00:30" to "time >22:30"). When the regression selected break points, marking changes in vocalization rate, at >02:30, >14:30, and >22:30, we shifted the day end to >02:30 and reran the regression in order to test the >22:30 break point.

Results and Discussion

Regression analyses showed two distinct ($p < 0.01$) daily levels of vocalization rate over a 4-d period at site 61. Between 04:30 and 08:30, the rate rose from about 7 calls/min to about 19 calls/min. It stayed at that level until after 16:30, and then dropped to about 7 calls/min again by midnight (Fig. 2). Because the recordings were 2 h apart, the break points cannot be fixed more accurately than 04:30–06:30 and 16:30–18:30. The regression analyses of the helicopter survey did not select time of day, but since we recorded all but three of the sites between 08:30 and 16:30, when the vocalization rate at site 61 was high and quite constant, we would expect a diel cycle to have little effect at the other sites, too.

Table 1 lists the data for the helicopter survey of each site. Segment number was not chosen by the regression, nor did a Kruskal-Wallis test, which does not assume normal distribution, show any difference in vocalization rates by 5-min segment ($H = 0.58$, df = 3, $p > 0.1$). If noise on the ice surface just before recording influenced ringed seal vocalization rates, the effect was not apparent in a 20-min recording, and a 10-min recording, as was used in previous studies, would give an equally good measure of vocalization rate.

Based on regression results, the degree of noise from ice, wind, or tape hiss on a recording did not measurably affect the number of vocalizations counted. These noises may mask

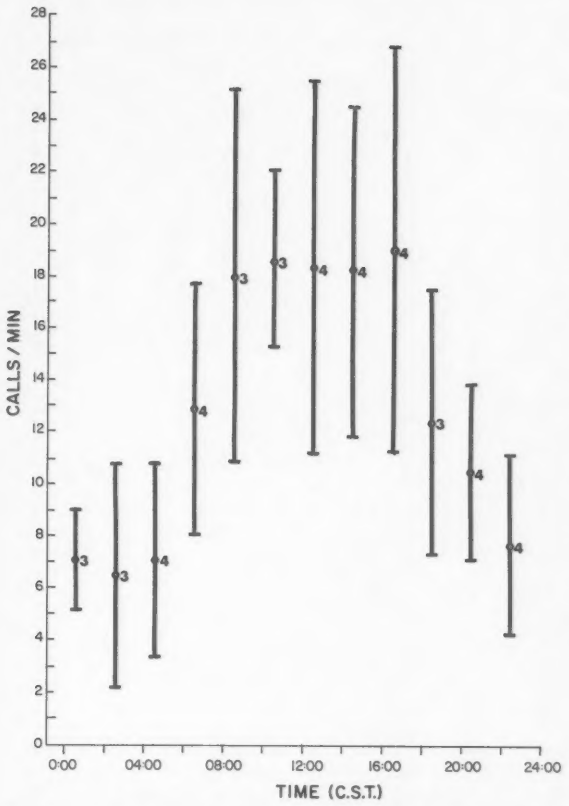

FIG. 2. Mean daily ringed seal vocalization rates at site 61, 23–27 April 1982 (±SD). Sample size is given by each point.

ringed seal vocalizations, but during this survey they were low enough not to affect our results.

The mean vocalization rate for all 32 sites was 15.7 calls/min (SD = 11.1). Analyses for site differences showed little variation in rate among the 32 sites; only 5 sites were significantly different from the mean ($p < 0.01$): sites 18 and 98 in Wellington Channel with >40 calls/min and sites 12, 97, and 99 deep in bays with <2 calls/min. The vocalization rate at each site is depicted using categories based on the analyses (Fig. 1).

Ice habitat class and snow depth were correlated with vocalization rates: sites in interisland channels, where there is less snow and the rough ice is unevenly distributed, have significantly higher than average vocalization rates, whereas sites in bays have deeper though less drifted snow and significantly lower vocalization rates ($p < 0.01$). It may be that our method of taking the average snow depth near a site did not accurately reflect the variability and patchiness of snow cover. The values for snow depth in bays varied much less than those in the other two habitat types (Table 1). The birth lair index, which we thought would be the best indicator of density and therefore vocalization rate, was never chosen by the regressions. This may have occurred because there was not enough spread in the values we assigned to the variable. We classified no habitat as 4 or 5, such as had been found in Amundsen Gulf (Smith and Stirling 1975). Except for site 61, we found only types 1 and 2.

Mean vocalization rates were similar between years ($p > 0.05$) for the 10 sites that were also surveyed in late April 1981 (Stirling et al. 1983, fig 6; Fig 1) ($\bar{x} = 18.0$, SD = 11.5 and $\bar{x} = 16.2$, SD = 11.0 for 1981 and 1982, respectively). There were some differences at individual sites, especially site 27, which is affected by the proximity of the open water in Lancaster Sound.

At Aston, Maxwell, and Radstock bays, where we had two or more recording sites, the vocalization rate at the mouths ($\bar{x} = 11.6$, SD = 2.3, $n = 3$) was almost three times the rate deep in the bays ($\bar{x} = 4.0$, SD = 2.8, $n = 5$). Site 12 in Freeman's Cove also had very low vocalization rates on this survey and in late March 1981 (Stirling et al. 1983). Polar bears observed hunting seals in Radstock and Maxwell bays in April concentrated their efforts in the drifted rough ice at the mouths of the bays and spent little time hunting deeper in the bays (Stirling and Latour 1978). Smith et al. (1978) also reported high densities of ringed seal birth lairs at the mouth of Aston Bay (site 96). From these data, we predict relatively low densities of seals deep in the bays during winter and early spring. However, high densities during summer have been reported in Freeman's Cove, Aston Bay, Radstock Bay, and Maxwell Bay (Smith et al. 1978; Finley 1979; Kingsley et al. 1985; I. Stirling, unpubl. data). Smith et al. (1978) and Finley (1979) thought that the change in density occurred because seals moved into these bays during breakup in late spring and summer. At that time, polar bears have been observed hunting throughout the smooth inner ice of Radstock and Maxwell bays (Stirling 1974; Stirling and Latour 1978). The magnitude of this change in the distributon of hunting polar bears supports our conclusion about low numbers of ringed seals deep in the bays during winter.

In southeastern Baffin Island and northwestern Amundsen Gulf, much of the preferred breeding habitat for ringed seals is deep in bays or where snow accumulates into deep drifts (McLaren 1958; Smith and Stirling 1975). In contrast, the High Arctic has much less snow, with drifts forming only where there are windbreaks such as pressure ridges. The snow in the flat, featureless bays is deeper than in coastal and channel areas, but is too shallow for lairs. Drifts form in the pressure ridges across the mouths of bays, along the coastlines of the main islands, and, to varying degrees, where rough ice occurs further offshore in areas such as northwestern Barrow Strait and southern Wellington Channel.

Drifted pressure ridges in the offshore areas of the wide interisland channels appear to be more important for ringed seal pupping in the High Arctic than in similar areas in southeastern Baffin Island or the eastern Beaufort Sea (Smith and Stirling 1975; Smith et al. 1978). As expected, we recorded average or higher vocalization rates at the sites near Griffith and Lowther islands, where relatively good birth lair habitat is consistently found in spring (Smith et al. 1978) and where high densities of ringed seals are observed hauled out in early summer (Kingsley et al. 1985). Sites near these two large islands, and sites 56 and 66 near small islands nearby, have a series of associated pressure ridges that contain some of the best birth lair habitat located to date in the High Arctic (Smith et al. 1978). However, the vocalization rates at these sites were similar to those at sites in the offshore areas in the middle of Barrow Strait, south of Lowther Island, and in southern Wellington Channel, which were judged by us and Stirling et al. (1983) to be poor habitat for birth lairs. Stirling et al. (1983) also reported high vocalization rates in these latter areas and suggested that central Barrow Strait and Wellington Channel likely contain substantial densities of immature and nonbreeding ringed seals throughout the year. The relatively high vocalization rate recorded in northern Peel Sound (site 87), which we classified as birth lair habitat 1,

TABLE 1. Ringed seal vocalization rates and habitat data collected for each site, 23–30 April 1982.

Site No.	Date in April	Start time	Calls/5-min segment 1	2	3	4	Calls/min	Noise[a] Ice	Wind	Tape	Mean snow depth ± SD (mm)	Ice habitat class[b]	Birth lair index[c]
8	27	13:19	13	22	25	53	5.6	3	1	2	296±104	B	1
9	27	12:20	26	53	30	40	7.4	3	1	2	409±85	B	1
10	27	11:06	68	53	70	33	11.2	3	1	2	270±204	C	1
11	23	11:35	74	57	68	79	13.9	0	0	2	153±159	I	2
12	24	10:12	6	2	0	11	1.0	3	0	0	246±104	B	1
16	26	15:08	103	81	56	93	16.6	1	1	3	93±57	I	1
17	30	12:47	25	26	26	15	4.6	3	0	0	451±81	B	1
18	27	08:19	228	169	310	185	44.1	2	1	3	89±59	I	1
26	23	12:40	85	129	238	102	27.7	0	0	2	136±94	I	2
27	27	14:56	81	35	41	33	9.5	2	0	0	191±105	C	2
53	23	09:16	84	81	80	153	19.9	3	0	3	206±129	C	1
56	23	17:57	49	121	201	199	28.5	3	0	0	158±246	I	2
57	24	12:30	101	52	79	33	13.2	2	0	0	205±248	C	1
59	24	11:14	51	34	28	73	9.3	3	0	0	170±146	I	1
61	23	10:30	50	136	101	142	21.4	0	0	2	144±98	I	3
62	28	15:44	50	111	27	40	11.4	0	0	3	147±110	C	1
64	26	11:58	146	0	11	1	7.9	1	1	3	149±134	C	2
65	28	14:40	86	93	72	64	15.8	0	0	0	138±108	C	2
66	23	16:55	52	28	74	77	11.6	0	0	3	238±155	I	2
67	26	08:52	24	28	23	37	5.6	1	1	2	128±140	C	2
70	24	09:03	22	93	69	39	11.2	3	0	0	130±120	I	1
87	24	15:45	156	144	153	20	23.6	3	0	2	195±108	I	1
88	24	14:33	115	149	257	59	29.0	2	0	0	119±114	I	2
89	24	13:34	108	135	124	55	21.1	2	0	0	217±221	I	2
90	26	09:59	43	38	27	26	6.7	2	1	3	248±294	I	2
91	26	10:57	79	40	55	74	12.4	2	1	3	143±102	I	1
92	26	14:01	189	99	66	192	27.3	1	1	2	84±63	I	1
93	26	12:56	136	136	171	67	25.5	1	1	2	72±41	I	1
96	28	12:58	90	63	43	84	14.0	3	0	0	198±139	C	1
97	28	13:46	3	2	1	7	0.6	2	0	3	202±87	B	1
98	27	09:13	260	305	157	150	43.6	2	1	2	69±40	I	1
99	30	13:23	9	2	12	11	1.7	3	0	0	309±98	B	1

[a]0, not estimated; 1, low; 2, medium; 3, high.
[b]B, bay; C, coastal rough ice; I, interisland channel.
[c]See Methods for categories.

is interesting, since Smith et al. (1978) reported high densities of ringed seal birth lairs there. This tends to support the idea that the distribution of suitable birth lair habitat may vary considerably between years in the offshore areas.

Site 70 at the south end of McDougall Sound and site 59 on the southeast coast of Bathurst Island had low vocalization rates. Site 59 also had a low rate in late April 1981 (Fig 1). Both areas are fairly shallow, had low densities of ringed seals during aerial surveys, and appear to be more favorable for bearded seals than for ringed seals.

Site 62 is the most disturbed location in the study area, from Inuit hunting parties, other traffic from oversnow machines, and low-level aircraft. Despite this, vocalization rates, although below average, are not very different from similar but less disturbed areas further west. Hunting and oversnow travel also occur near sites 53 and 67. The vocalization rate at site 53 is higher than the overall survey average, while it is lower at the latter. The rates at these sites are consistent with data from 1981 (Stirling et al. 1983; Fig. 1). Although our sample size is small, it does not appear that these kinds of disturbance have a detrimental effect that we can determine by measuring vocalization rates.

From dog searches (Smith and Stirling 1975), we know that high vocalization rates are found in areas with good pupping habitat, i.e. areas of rough ice with snow drifts deep enough for birth lairs, yet our measurements of habitat variables at each recording site were poor predictors of vocalization rate. It may be that better pupping habitat under deep snow in the rough ice and channels is partitioned into territories. Thus, seal density is lower there than in less favorable pupping habitat with less snow occupied by nonbreeders and immatures. It is also possible that areas occupied by nonbreeders have more vocalizations because a dominance hierarchy has not been established, and vocalization rate reflects not only density but also social structure. Then, our assumption that a high vocalization rate indicates the best pupping habitat and highest densities would not hold true.

We conclude that whereas vocalization rates have some use in the assessment of relative abundance of wintering ringed seals, the vocalization rates in different habitats overlap too much to be used as a diagnostic character for separating suitable and unsuitable pupping areas. To date, the dog-search technique remains the only effective way to determine the distribution of ringed seals in the winter and the distribution of their birth lairs in the early spring.

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References

CANADIAN ARCTIC RESOURCES COMMITTEE. 1980. Marine traffic forecast, Northwest Passage. Appendix to Canadian Arctic Resources Committee submission to EARP review of the Arctic Pilot Project. April 1980. Ottawa. 71 p. Available from CARC, 46 Elgin St., Ottawa, Ont. K1P 5K6.

DRAPER, N. R., AND H. SMITH. 1981. Applied regression analysis. 2nd ed. J. Wiley & Sons Inc., New York, NY. 709 p.

FINLEY, K. J. 1979. Haul-out behaviour and densities of ringed seals (*Phoca hispida*) in the Barrow Strait area, N.W.T. Can. J. Zool. 57: 1985–1997.

KINGSLEY, M. C. S., I. STIRLING, AND W. CALVERT. 1985. The distribution and abundance of seals in the Canadian high Arctic, 1980–82. Can. J. Fish. Aquat. Sci. 42: 1189–1210.

MCLAREN, I. A. 1958. The biology of the ringed seal (*Phoca hispida*) in the eastern Canadian Arctic. Bull. Fish. Res. Board Can. 118: 97 p.

SMITH, T. G., AND M. O. HAMMILL. 1981. The ecology of the ringed seal in its fast ice breeding habitat. Can. J. Zool. 59: 966–981.

SMITH, T. G., K. HAY, D. TAYLOR, AND R. GREENDALE. 1978. Ringed seal breeding habitat in Viscount Melville Sound, Barrow Strait and Peel Sound. ESCOM Report No. AI-22. Department of Indian and Northern Affairs, Ottawa, Ont. K1A 0H4. 85 p.

SMITH, T. G., AND I. STIRLING. 1975. The breeding habitat of the ringed seal (*Phoca hispida*): the birth lair and associated structures. Can. J. Zool. 53: 1297–1305.

1978. Variation in the density of ringed seal (*Phoca hispida*) birth lairs in the Amundsen Gulf, Northwest Territories. Can. J. Zool. 56: 1066–1070.

STIRLING, I. 1974. Midsummer observations on the behavior of wild polar bears (*Ursus maritimus*). Can. J. Zool. 52: 1191–1198.

STIRLING, I., AND W. CALVERT. 1983. Environmental threats to marine mammals in the Canadian Arctic. Polar Rec. 21: 433–449.

STIRLING, I., W. CALVERT, AND H. CLEATOR. 1983. Underwater vocalizations as a tool for studying the distribution and relative abundance of wintering pinnipeds in the High Arctic. Arctic 36: 262–274.

STIRLING, I., H. CLEATOR, AND T. G. SMITH. 1981. Marine mammals, p. 45–58. *In* I. Stirling and H. Cleator [ed.] Polynyas in the Canadian Arctic. Can. Wildl. Serv. Occas. Pap. 45.

STIRLING, I., AND P. B. LATOUR. 1978. Comparative hunting abilities of polar bear cubs of different ages. Can. J. Zool. 56: 1768–1772.

STIRLING, I., R. E. SCHWEINSBURG, W. CALVERT, AND H. P. L. KILIAAN. 1978. Population ecology of the polar bear along the proposed Arctic Islands gas pipeline route. ESCOM Report No. AI-24. Department of Indian and Northern Affairs, Ottawa, Ont. K1A 0H4. 93 p.

Use of a Bacterial Bioluminescence Assay to Assess Toxicity of Contaminated Marine Sediments

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A bacterial bioluminescence assay was evaluated for assessing the toxicity of contaminated marine sediments. Preliminary assays established the feasibility of testing methanol–dichloromethane sediment extracts and demonstrated the advantages of solvent-exchanging the extracts into ethanol before testing. Bioluminescence assays were then conducted on extracts of 18 marine sediments varying in nature and degree of chemical contamination. Statistical analyses revealed significant associations between acute toxicity expressed as a 15-min EC50 (the concentration of extract causing a 50% reduction in bioluminescence after a 15-min exposure) and concentrations of sums of measured aromatic hydrocarbons, naphthalenes, and chlorinated hydrocarbons. We conclude that the bioluminescence assay is useful as a rapid method of comparing and ranking the toxicity of organic extracts of contaminated sediments.

Les auteurs ont évalué un test de bioluminescence bactérienne pour quantifier la toxicité de sédiments marins contaminés. Des tests préliminaires ont établi la faisabilité de la vérification d'extraits de sédiments au méthanol–dichlorométhane et ont révélé les avantages de remplacer le solvant des extraits par de l'éthanol avant les essais. On a alors mené des tests de bioluminescence sur des extraits de 18 sédiments marins contaminés à divers degrés par une variété de produits chimiques. Des analyses statistiques ont révélé de nettes associations entre la toxicité aiguë exprimée en CE50 15 min (c.-à-d. la concentration d'extrait entraînant une baisse de 50 % de la bioluminescence après une exposition de 15 min) et les concentrations totales quantifiées d'hydrocarbures aromatiques, de naphtalène et d'hydrocarbures chlorés. Les auteurs formulent la conclusion que le test de bioluminescence est utile comme méthode rapide pour la comparaison et la classification de la toxicité des extraits organiques de sédiments contaminés.

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Sediments are a primary repository for anthropogenic chemicals of limited solubility entering the marine environment (Means et al. 1979). Major sources of these xenobiotics are surface runoff, atmospheric fallout, and industrial and domestic sewage discharge. Included among these myriad chemicals are metals, aromatic hydrocarbons, and chlorinated hydrocarbons, which are known to be acutely toxic and, in selected organisms, carcinogenic. Also present are numerous unidentified compounds of unknown toxicity (Malins et al. 1984). Although often tightly sorbed to sediment particulates, the presence of many of these compounds or their metabolites in marine organisms clearly demonstrates their bioavailability (Krahn et al. 1984; Malins et al. 1982; Rubinstein et al. 1983).

A variety of biological assays have been used to assess the toxicity of contaminated marine sediments. Two of the more promising test systems have been the amphipod (*Rhepoxynius abronius*) lethality bioassay (Swartz et al. 1982) and the Pacific oyster (*Crassostrea gigas*) embryo lethality and shell development bioassay (Weolke 1972; Chapman and Morgan 1983). Bacteria are now also widely employed in studies of the toxic effects of environmental contaminants (Bitton 1983); however, their use with sediments and sediment-associated contaminants has been limited. Several short-term microbial assays have undergone extensive evaluation with aqueous toxicants and results compared quite favorably with those obtained using *Daphnia* sp., fathead minnows (*Pimephales promelas*), and rainbow trout (*Salmo gairdneri*) (Bulich et al. 1981; Lebsack et al. 1981; Curtis et al. 1982; Qureshi et al. 1982; DeZwart and Slooff 1983). Moreover, microorganisms offer several distinct advantages over these more traditional test species. Bacteria can be readily maintained and cultured in the laboratory; they grow rapidly so toxic effects can be measured over several generations in a short time period; and because of their ability to grow at high densities (approximately 10^9/mL), testing large populations of organisms takes a minimum of space.

In this study we evaluate the use of a bacterial bioluminescence assay (Bulich 1979) to assess the toxicity of marine sediments. The bioluminescence assay is a rapid, sensitive method of toxicity testing based on light emission by luminescent bacteria in the presence and absence of aqueous toxicants. The emitted light is a product of the electron transport system (Hastings and

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Nealson 1977) which directly reflects the metabolic state of the cell. Here we report the toxicity of organic extracts prepared from 18 marine sediments varying in nature and degree of contamination.

Materials and Methods

Sediment Collection and Analyses

Sediment samples were collected from 18 locations in Puget Sound, Washington, using a modified 0.1-m^2 Van Veen sediment grab (Word 1976). About 500 g of sediment from the top 2 cm was transferred to dichloromethane-rinsed glass jars which were sealed with Teflon sheeting and placed on ice. Samples were frozen at −20°C within 6 h of collection and stored frozen until extracts were prepared. Dry weight was determined by heating a 10-g aliquot to a constant weight at 150°C.

Sediments were extracted as previously described by MacLeod et al. (1984). Sediments were thawed and 100-g samples were washed with methanol to remove water and then extracted with 2:1 dichloromethane–methanol by tumbling in 250-mL glass bottles (twice for approximately 18 h and once for 6 h). The methanol washings and the dichloromethane–methanol extracts were combined and washed with water to remove the methanol and then dried over sodium sulfate. The resulting dichloromethane extract was concentrated to 25.0 mL. A 100-μL portion was added to 3.0 mL of the solvent vehicle and concentrated to approximately 2.0 mL to remove the dichloromethane. The volume was returned to 3.0 mL, and this standardized solution of extract was used in the bioluminescence assays. Extraction blanks were prepared by an identical procedure but without sediment.

The presence of aromatic and chlorinated hydrocarbons in sediment extracts was determined by gas chromatography as described by MacLeod et al. (1984). Flame ionization detection was employed for aromatic hydrocarbons, electron capture detection for chlorinated hydrocarbons, and gas chromatography / mass spectrometry for the identity of the compounds. Compounds were quantitated using internal standards.

Although the extraction procedure we used presumably excluded the toxic metals often present in relatively high concentrations in the sediments of urban waterways (Malins et al. 1982), their presence in the extracts as part of organic complexes could not be ruled out. Accordingly, nitric acid digests of selected sediment extracts were subjected to plasma emission spectrographic analyses. While detectable, albeit low, concentrations of copper, magnesium, potassium, selenium, sodium, and zinc were present, no arsenic, cadmium, chromium, mercury, or lead was detected. For example, the concentrations of detected metals in the extract of Duwamish-A sediment (one of the most contaminated sediments tested) were all less than 0.1 μg/mL with the exception of sodium which was present at a concentration of 0.9 μg/mL.

Solvent Vehicles and Bioluminescence Assays

Selected organic solvents of the highest purity commercially available were evaluated for use in the bioluminescence assays as the extract vehicle. Methanol, acetone, dimethyl sulfoxide (DMSO), and dichloromethane were obtained from Burdick and Jackson Laboratories, Muskegon, MI. Ethanol was USP-grade from Warner-Grahm, Cockeyville, MD. Solvents were exchanged by the procedure described by MacLeod et al. (1984) prior to the toxicity testing of extracts.

TABLE 1. Summary of bacterial bioluminescence tests of selected organic solvents screened for use in sediment extract toxicity bioassays. Results are expressed as estimates of the 15-min EC50s and their 95% confidence intervals.

Solvent	Toxicity (15-min EC50 and 95% CI, μL/mL)
Dichloromethane	2.150 (1.981–2.328)
Acetone	18.35 (14.24–26.76)
Methanol	18.62 (11.18–19.94)
Ethanol	29.11 (23.01–35.00)
DMSO	58.39 (55.00–62.24)

Bacterial bioluminescence assays were conducted essentially as described by Bulich and Isenberg (1981). Briefly, saline suspensions containing approximately 10^6 colony-forming units (CFU) of the luminescent bacterium *Photobacterium phosphoreum* (NRRL B11177) were exposed for up to 30 min at 15°C to selected concentrations of sediment extracts or solvent vehicles. Bioluminescence was monitored at 0-time and after 5, 15, and 30 min of exposure using a model 2055 Microtox Toxicity Analyzer (Beckman Instruments, Carlsbad, CA).[3]

Routinely, four concentrations of extracts or solvents and a saline blank were tested in duplicate. Prior to testing, extract and solvent samples were adjusted to contain 2% NaCl. A saline solution consisting of 2% NaCl (Mallinckrodt, USP grade) prepared with double-distilled, charcoal-filtered water was used for all dilutions. Working solutions of test bacteria were prepared daily by reconstituting a lyophilized suspension of *P. phosphoreum* (Microtox reagent, Beckman Instruments, Carlsbad, CA) in sterile, charcoal-filtered, double-distilled water. This suspension, containing approximately 10^8 CFU/mL, was held at 3 ± 1°C and was used for up to 8 h after reconstitution. A reference toxicant consisting of 20% ethanol in 2.0% NaCl was tested each day to quantitate test variability.

Estimates of the EC50 (the concentration of extract causing a 50% reduction in bioluminescence) were obtained using linear regression analysis. The percent inhibition of light emitted at each test concentration and time point was converted to a gamma value which has been defined as the ratio of light lost to light remaining (Johnson 1974). Gamma values were normalized for natural decline in light production measured over time as described by Bulich et al. (1981). For bioassays of sediment extracts, gamma values were also adjusted for the contribution of the solvent vehicle. The natural log of gamma was regressed on the natural log of extract concentration, and we calculated the EC50 from the regression equation. We used a mathematical procedure based on Fieller's theorem (Finney 1964) to calculate a 95% confidence interval for each estimate of the EC50. To test for an association between acute toxicity and selected chemical characteristics of the sediments, we used Spearman's rank correlation procedure.

Results

Results of bioluminescence assays evaluating the toxicity of selected solvents are shown in Table 1. Dichloromethane exhibited the greatest toxicity of those solvents tested, with an

[3]Mention of trade names is for information only and does not constitute endorsement by the U.S. Department of Commerce.

TABLE 2. Toxicity of DMSO and DMSO amended with 10% dichloromethane after incubation for up to 120 min at room temperature and 110°C. Results are expressed as estimates of the 15-min EC50s and their 95% confidence intervals.

Incubation time (min)	Toxicity (15-min EC50 and 95% CI, μL/mL)			
	DMSO	DMSO (heated)	DMSO + 10% CH_2Cl_2	DMSO + 10% CH_2Cl_2 (heated)
0	57.60 (54.26–61.37)	57.17 (51.42–64.24)	14.41 (11.50–19.08)	13.57 (12.21–15.25)
30	58.26 (55.36–61.49)	60.61 (54.58–68.20)	13.12 (12.46–13.81)	13.13 (11.76–14.83)
60	59.65 (49.57–74.36)	51.30 (47.47–55.72)	14.02 (12.98–15.24)	2.510 (2.423–2.692)
120	54.80 (51.76–58.20)	52.89 (49.92–56.20)	13.32 (12.25–14.60)	0.6163 (0.5440–0.7123)

estimated 15-min EC50 of 2.2 μL/mL (parts per thousand, ppt). On the other end of the spectrum were DMSO and ethanol with estimated 15-min EC50s of 58.4 and 29.1 ppt, respectively. Based on these results we concluded that the solvent in which the extracts were originally dissolved (dichloromethane) needed to be replaced by (solvent-exchanged to) one of lesser toxicity before testing and, moreover, that DMSO and ethanol were of obvious potential.

To further evaluate the usefulness of DMSO and ethanol as solvent vehicles, we ran bioluminescence assays on extraction blanks exchanged from dichloromethane into both prospective solvents. The results showed that the toxicity of DMSO increased approximately 14 times after the solvent exchange. The estimated 15-min EC50s for exchanged versus nonexchanged DMSO were 4.1 (3.6–4.5) and 56.1 (51.2–63.1) ppt, respectively. In contrast, exchange of an extraction blank into ethanol caused little change in the toxicity of the receiving solvent. Estimated 15-min EC50s for exchanged versus nonexchanged ethanol were 33.8 (26.9–42.9) and 33.0 (26.6–39.4) ppt, respectively.

A series of additional assays was conducted to identify factors contributing to the increased toxicity observed when an extraction blank was exchanged into DMSO. One obvious possibility was the presence of trace amounts of dichloromethane resulting from incomplete solvent exchange. This was apparently not the case, as the estimated 15-min EC50 of DMSO amended with 3.3% dichloromethane (the maximum calculated concentration that could have been present) was 53.2 (46.6–61.7) ppt, an estimate not significantly different from that of unamended DMSO. Moreover, neither incubating DMSO at room temperature nor heating (approximately 110°C), both for up to 120 min, resulted in a major change in toxicity (Table 2). However, when DMSO was heated in the presence of 10% dichloromethane, the toxicity rapidly increased until, at 120 min, the toxicity of the heated mixture was about 22 times greater than an identical solution incubated at room temperature. These results suggested that, at elevated temperature, a new highly toxic compound(s) had formed.

In contrast with the marked change in toxicity of DMSO observed after solvent exchange with dichloromethane, exchanges from dichloromethane to ethanol were relatively straightforward. The toxicity of ethanol after exchange with dichloromethane was not significantly different from unexchanged ethanol (Table 3). We also found that exchanging dichloromethane first into ethanol and then into DMSO resulted in a minimal change in the toxicity of the DMSO. Nonetheless, we selected ethanol as the solvent vehicle for sediment extracts due to its direct exchange potential and relatively low toxicity.

TABLE 3. Toxicity of DMSO and ethanol alone, and after solvent exchange. Results are expressed as estimates of the 15-min EC50s and their 95% confidence intervals.

Test material	Toxicity (15-min EC50 and 95% CI, μL/mL)
DMSO	56.43 (51.18–63.06)
EtOH	33.41 (28.49–39.17)
EtOH heated for 1 h	25.42 (22.88–28.03)
CH_2Cl_2 replaced by EtOH by heating 1 h	30.34 (24.59–38.83)
CH_2Cl_2 replaced by EtOH by heating 1 h; EtOH replaced by DMSO by heating 1 h	46.46 (43.25–50.05)

The results of bioluminescence assays of organic extracts of sediments collected at 18 sites in Puget Sound and results of chemical analyses of the sediment extracts for aromatic and chlorinated hydrocarbons are shown in Table 4. Acute toxicity ranged from a high of 0.14 ppt for the extract prepared from Hylebos Waterway sediment to a low of 14.48 ppt for the extract of Clinton sediment. The sums of the measured aromatic hydrocarbons ranged from 110 to 310 000 ng/g for extracts of Clinton and Eagle Harbor-D sediments, respectively. Concentrations of naphthalenes (sum of the concentrations of naphthalene, 1-methynaphthalene, 2-methylnaphthalene, 2,6-dimethylnaphthene, and 2,3,5-trimethylnaphthalene), which serve as representatives of the more toxic low molecular weight polynuclear aromatic hydrocarbons, ranged from 24 000 ng/g in the Eagle Harbor-D extract down to nondetectable levels in the Clinton extract. Chlorinated hydrocarbons were detected in only 8 of the 18 sediment extracts, with the highest concentration, 4600 ng/g, found in the extract prepared from Hylebos Waterway sediment.

Statistical evaluation of these data revealed a highly significant association ($p = 0.001$) between acute toxicity and sums of the concentrations of aromatic hydrocarbons ($r_s = 0.828$)

TABLE 4. Comparison of toxicities of organic extracts of selected Puget Sound sediments and sums of the measured concentrations of aromatic hydrocarbons (AHs), chlorinated hydrocarbons (CHs), and naphthalenes (BLD = below limits of detection).

Source of sediment[a]	Acute toxicity[b]	AHs (ng/g)	CHs (ng/g)	Naphthalenes (ng/n)[c]
Hylebos Waterway	0.14 (0.13–0.16)	22 000	4600	450
Duwamish-A	0.20 (0.18–0.22)	27 000	1200	540
Eagle Harbor-D	0.25 (0.22–0.29)	310 000	BLD	24 000
Everett Harbor	0.25 (0.24–0.26)	5 600	46	1 700
Discovery Bay	0.36 (0.34–0.39)	810	BLD	25
Eagle Harbor-C	0.44 (0.37–0.52)	14 000	47	480
Eagle Harbor-B	0.51 (0.45–0.57)	10 000	42	340
Duwamish-B	1.30 (1.12–1.49)	2 600	340	210
West Point	2.18 (1.80–2.55)	34 000	14	780
Eagle Harbor-E	2.95 (2.61–3.39)	5 200	BLD	420
Eagle Harbor-F	5.64 (4.88–6.87)	2 000	BLD	59
President Point	5.69 (5.25–6.15)	1 100	BLD	8.9
Eagle Harbor-A	6.18 (4.93–8.51)	1 600	4.7	150
Useless Bay	7.37 (6.44–8.25)	130	BLD	11
Edmonds	8.77 (7.14–10.43)	650	BLD	40
Richmond Beach	10.66 (9.53–11.87)	290	BLD	23
Carkeek Park	12.45 (10.54–14.63)	220	BLD	BLD
Clinton	14.48 (11.78–18.22)	110	BLD	BLD

[a]Sediments designated by location; letters are independent samples collected in the same general vicinity.

[b]15-min EC50 and 95% confidence interval (μL/mL).

[c]Sums of the concentrations of naphthalene, 1-methylnaphthalene, 2-methylnaphthalene, 2,6-dimethylnaphthalene, and 2,3,5-trimethylnaphthalene.

and between acute toxicity and sums of the concentrations of naphthalenes ($r_s = 0.796$). A significant association ($P = 0.005$) was also demonstrable between acute toxicity and sum of the concentrations of chlorinated hydrocarbons ($r_s = 0.669$).

Discussion

Although the bacterial bioluminescence assay has been frequently used in toxicity testing of aqueous effluents and water-soluble compounds, its use with contaminated sediments has received limited attention. Based on the results presented here, we conclude that it is also a useful method for testing and comparing the toxicity of organic extracts of marine sediments.

We first established the feasibility of testing organic extracts in a series of experiments screening potential solvent vehicles and exchanging extraction blanks from dichloromethane into ethanol and DMSO. While the results clearly indicated that ethanol was the solvent vehicle of choice, the demonstration of greatly enhanced toxicity of DMSO after exchange with dichloromethane was also noteworthy. Although subsequent testing suggested that the enhanced toxicity was due to the formation of a new compound(s), no attempt was made to identify such products. These observations indicate that DMSO, a solvent vehicle frequently used in toxicity testing of organic compounds of limited solubility, should be used with considerable caution.

We evaluated the bioluminescence assay by testing the toxicity of organic extracts of 18 marine sediments and demonstrated significant associations between acute toxicity and the degree of contamination with aromatic and chlorinated hydrocarbons. Although the highest correlation was observed when acute toxicity was related to the sums of the concentrations of all measured hydrocarbons, significant associations were also demonstrable with the sums of the concentrations of naphthalenes and chlorinated hydrocarbons. Sediments containing elevated levels of aromatic and chlorinated hydrocarbons collected near urban areas, however, have a high likelihood of contamination with additional, and often unidentified, anthropogenic chemicals (Malins et al. 1984). Although the organic extracts we used were shown not to contain appreciable levels of metals, it would be indefensible to attribute toxicity to aromatic and chlorinated hydrocarbons only. Nonetheless, the results clearly show the utility of the bioluminescence bioassay for comparing and ranking sediments based on the toxicity of organic extracts. Moreover, our findings strongly support the use of this assay as one of a battery of biological tests used in any comprehensive evaluation of the toxicity of contaminated sediments.

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References

BITTON, G. 1983. Bacterial and biochemical tests for assessing chemical toxicity in the aquatic environment: a review. CRC Crit. Rev. Environ. Control 13: 51–67.

BULICH, A. A. 1979. Use of luminescent bacteria for determining toxicity in aquatic environments, p. 98–106. *In* L. L. Marking and R. A. Kimerle [ed.] Aquatic toxicology. ASTM STP 667. American Society for Testing and Materials, Philadelphia, PA.

BULICH, A. A., M. W. GREENE, AND D. L. ISENBERG. 1981. Reliability of the bacterial luminescence assay for determination of the toxicity of pure compounds and complex effluents, p. 338–347. *In* D. R. Branson and K. L. Dickson [ed.] Aquatic toxicology and hazard assessment. ASTM STP 737. American Society for Testing and Materials, Philadelphia, PA.

BULICH, A. A., AND D. L. ISENBERG. 1981. Use of the luminescent bacterial system for the rapid assessment of aquatic toxicity. Trans. Am. Inst. Soc. 20: 29–33.

CHAPMAN, P. M., AND J. D. MORGAN. 1983. Sediment bioassays with oyster larvae. Bull. Environ. Contam. Toxicol. 31: 438–444.

CURTIS, C., A. LIME, S. J. LOZANO, AND G. D. VEITH. 1982. An evaluation of a bacterial bioluminescence bioassay as a method for predicting acute toxicity of organic chemicals to fish, p. 170–178. *In* J. G. Pearson, R. B. Foster and W. E. Bishop [ed.] Aquatic toxicology. ASTM STP 766. American Society for Testing and Materials, Philadelphia, PA.

DEZWART, D., AND W. SLOOFF. 1983. The microtox as an alternative assay in the acute toxicity assessment of water pollutants. Aquat. Toxicol. 4: 129–138.

FINNEY, D. J. 1964. Statistical methods in biological assay. Charles Griffin & Company Limited, London. 668 p.

HASTINGS, J. W., AND K. H. NEALSON. 1977. Bacterial bioluminescence. Annu. Rev. Microbiol. 31: 549–595.

JOHNSON, F. H. 1974. The theory of rate process in biology and medicine. John Wiley & Sons, New York, NY. 730 p.

KRAHN, M. M., M. S. MYERS, D. G. BURROWS, AND D. C. MALINS. 1984. Determination of metabolites of xenobiotics in the bile of fish from polluted waterways. Xenobiotica 14: 633–646.

LEBSACK, M. E., A. D. ANDERSON, G. M. DEGRAEVE, AND H. L. BERGMAN. 1981. Comparison of bacterial luminescence and fish bioassay results for fossil-fuel process waters and phenolic constituents, p. 348–356. *In* D. R. Branson and K. D. Dickson [ed.] Aquatic toxicology and hazard assessment. ASTM STP 737. American Society for Testing and Materials, Philadelphia, PA.

MACLEOD, W. D., D. BROWN, A. FRIEDMAN, O. MAYNES, AND R. PEARCE. 1984. Standard analytical procedures of the NOAA National Analytical Facility — Extractable Toxic Organic Compounds. U.S. Dep. Commer., NOAA Tech. Memo. NMFS, F/NWC-64: 110 p. (Available from U.S. Department of Commerce, National Technical Information Service, Springfield, VA)

MALINS, D. C., B. B. MCCAIN, D. W. BROWN, S. L. CHAN, M. S. MYERS, J. T. LANDAHL, P. G. PROHASKA, A. J. FRIEDMAN, L. D. RHODES, D. G. BURROWS, W. D. GRONLUND, AND H. O. HODGINS. 1984. Chemical pollutants in sediments and diseases of bottom-dwelling fish in Puget Sound, Washington. Environ. Sci. Technol. 18: 705–713.

MALINS, D. C., B. B. MCCAIN, D. W. BROWN, A. K. SPARKS, H. O. HODGINS, AND S-L. CHAN. 1982. Chemical contaminants and abnormalities in fish and invertebrates from Puget Sound. U.S. Dep. of Commer., NOAA Tech. Memo. OMPA-19: 168 p. (Available from U.S. Department of Commerce, National Technical Information Service, Springfield, VA, as PB83-115188)

MEANS, J. C., J. J. HASSETT, S. G. WOOD, AND W. L. BANWART. 1979. Sorption properties of energy-related pollutants and sediments, p. 327–340. *In* P. W. Jones and P. Leber [ed.] Polynuclear aromatic hydrocarbons. Ann Arbor Science Publishers, Ann Arbor, MI.

QURESHI, A. A., K. W. FLOOD, S. R. THOMPSON, S. M. JANHURST, C. S. INNISE, AND D. A. ROKOSH. 1982. Comparison of a luminescent bacterial test with other bioassays for determining toxicity of pure compounds and complex effluents, p. 179–195. *In* J. G. Pearson, R. B. Foster, and N. E. Bishop [ed.] Aquatic toxicology. ASTM STP 766. American Society for Testing and Materials, Philadelphia, PA.

RUBINSTEIN, N. I., E. LORES, AND N. R. GREGORY. 1983. Accumulation of PCBs mercury and cadmium by *Nereis virens*, *Mercenaria mercenaria* and *Palaemonetes pugio* from contaminated harbor sediments. Aquat. Toxicol. 3: 249–260.

SWARTZ, R. C., W. A. DEBEN, K. A. SERCU, AND J. O. LAMBERSON. 1982. Sediment toxicity and the distribution of amphipods in Commencement Bay, Washington, U.S.A. Mar. Pollut. Bull. 13: 359–364.

WOELKE, C. E. 1972. Development of a receiving water quality bioassay criterion based on the 48-hour Pacific oyster, *Crassostrea gigas* embryo. Wash. Dep. Fish. Tech. Rep. 9: 93 p.

WORD, J. Q. 1976. Biological comparison of grab sampling devices. South. Calif. Coastal Water Res. Proj. Annu. Rep. 1976: 189–194.

Excess Unsupported ^{210}Pb in Lake Sediment from Rocky Mountain Lakes: A Groundwater Effect

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Norton, S. A., C. T. Hess, G. M. Blake, M. L. Morrison, and J. Baron. 1985. Excess unsupported ^{210}Pb in lake sediment from Rocky Mountain lakes: a groundwater effect. Can. J. Fish. Aquat. Sci. 42: 1249–1254.

Sediment cores from four high-altitude (approximately 3200 m) lakes in Rocky Mountain National Park, Colorado, were dated by ^{210}Pb chronology. Background (supported) ^{210}Pb activities for the four cores range from 0.26 to 0.93 Beq/g dry weight, high for typical oligotrophic lakes. Integrated unsupported ^{210}Pb ranges from 0.81 (a typical value for most lakes) to 11.0 Beq/cm^2. The ^{210}Pb activity in the surface sediments ranges from 1.48 to 22.2 Beq/g dry weight. Sediment from Lake Louise, the most unusual of the four, has 22.2 Beq/g dry weight at the sediment surface, an integrated unsupported ^{210}Pb = 11.0 Beq/cm^2, and supported ^{210}Pb = 0.74 Beq/g dry weight. ^{226}Ra content of the sediment is insufficient to explain either the high unsupported ^{210}Pb or the ^{222}Rn content of the water column of Lake Louise, which averaged 96.2 Beq/L. We concluded that ^{222}Rn-rich groundwater entering the lake is the source of the high ^{222}Rn in the water column. This, in turn, is capable of supporting the unusually high ^{210}Pb flux to the sediment surface. Groundwater with high ^{222}Rn may control the ^{210}Pb budget of lakes where sediment cores have integrated unsupported ^{210}Pb greater than 2 Beq/cm^2.

Des carottes de sédiments prélevées dans quatre lacs de haute altitude (env. 3200 m) du parc national Rocky Mountain (Colorado) ont été datées au Pb^{210}. L'activité ambiante du Pb^{210} dans les quatre carottes variait de 0,26 à 0,93 Beq/g poids sec, des valeurs élevées pour des lacs oligotrophes typiques, tandis que l'activité intégrée allait de 0,81 (une valeur typique pour la plupart des lacs) à 11,0 Beq/cm^2. Dans les sédiments superficiels, l'activité du Pb^{210} variait de 1,48 à 22,2 Beq/g poids sec. On a obtenu les valeurs suivantes pour les sédiments du lac Louise, le plus insolite des quatre : 22,2 Beq/g poids sec à la surface, une activité intégrée du Pb^{210} de 11,0 Beq/cm^2 et une activité ambiante du Pb^{210} de 0,74 Beq/g poids sec. La teneur des sédiments en Ra^{226} n'est pas suffisante pour expliquer l'activité intégrée élevée du Pb^{210} ou la teneur en Rn^{222}, qui s'élevait en moyenne à 96,2 Beq/L, dans la colonne d'eau du lac Louise. Les auteurs formulent la conclusion que les eaux souterraines riches en Rn^{222} qui se déversent dans le lac sont la source de la teneur élevée en Rn^{222} dans la colonne d'eau. Ceci peut suffire au flux exceptionnellement élevé de Pb^{210} à la surface des sédiments. Les eaux souterraines à forte teneur en Rn^{222} peuvent contrôler le bilan en Pb^{210} de lacs dont les carottes sédimentaires montrent une activité intégrée du Pb^{210} supérieure à 2 Beq/cm^2.

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The analysis of short sediment cores from lakes has been commonly employed to trace historical developments within the lake, including cultural entrophication, pollution, and most recently the acidification of lakes due to acidic precipitation. Associated with the latter process are changing fluxes of metals, including accelerated atmospheric deposition of metals such as Pb, Zn, and Cu (Norton and Kahl 1983) as well as accelerated mobilization of metals from the lake/drainage basin (Hanson et al. 1982). Of paramount importance in these studies is establishing the absolute age of the sedimentary strata for purposes of chronology of events recorded in the sediment record as well as enabling absolute flux calculations for metals.

At present, the use of ^{137}Cs and ^{210}Pb radionuclides for dating is most popular. Each technique has certain assumptions that must be satisfied for credible results. Recently, Davis et al.

TABLE 1. Limnologic characteristics of the four study lakes, Rocky Mountain National Park, Colorado.

	Elevation (m)	Catchment area (ha)	Catchment area: Lake area	Catchment area: Relief (m)	Coring depth (m)	Average depth (m)	Maximum depth (m)	Lake pH
Lake Husted	3380	107	10:1	316	8	3	8.3	6.87
Lake Louise	3365	111	40:1	303	6	2	6	6.75
Emerald Lake	3110	205	40:1	786	16	8	17	6.09–6.15
Lake Haiyaha	3110	404	30:1	762	8.5	7	10	6.26–6.46

(1984) have suggested that ^{137}Cs dating does not give reliable results in lakes that have soft water, are oligotrophic, have slightly acidic to acidic pH, and have sediments with little clay-mineral content. Our studies have focused on these types of lakes; consequently we have routinely used ^{210}Pb dating for our cores. However, this method is also not without problems, including bioturbation (Imboden and Stiller 1982), diffusion of ^{222}Rn (a precursor of ^{210}Pb) within the sediment column or out of it (Imboden and Stiller 1982), non-steady-state sediment deposition rates or chemistry (Norton 1983), non-steady-state sediment focusing and winnowing (Evans and Rigler 1983), non-steady-state flux of unsupported (by ^{226}Ra) ^{210}Pb to the sediment surface, and acidification of surface waters over the last 100 yr or less (Dillon and Smith 1983). In spite of these shortcomings, ^{210}Pb dating appears to give reasonable results in most cases when compared with other techniques of unequivocal reliability such as stratigraphic marker beds (e.g. pollen) and varves (see e.g. Johnston et al. 1982).

We report here on the ^{210}Pb analysis of sediment cores from four subalpine to alpine lakes. Our objective was to investigate the cause of the large flux of ^{210}Pb to the sediment and propose that the ^{210}Pb is supported by ^{222}Rn delivered to the lake via groundwater.

Materials and Methods

We studied two pairs of lakes. The Louise–Husted lake pair (Fig. 1) is located primarily on the Sherman Quartz Monzonite (Nesse 1972); the watersheds of Emerald and Haiyaha are underlain by biotite gneiss and schist, intruded by the Silver Plume granite (Johnson and Herzog 1983; Cole 1977). All the lakes are headwater tarns and clear water oligotrophic lakes. The watersheds consist primarily of active talus slopes, exposed bedrock, and thin to nonexistent soils. Vegetation is primarily alpine tundra with minor amounts of krumholtz. Permanent snow fields occupy part of the drainage of Lakes Louise, Emerald, and Haiyaha.

Sediment cores were taken from the deeper part of each lake (Table 1) in July 1982 with a 6.25-cm-diameter stationary piston corer (Davis and Doyle 1969). Sediment was extruded upward from the core tube and sectioned in the field in 0.5-cm increments from 0 to 20 cm and in 1.0-cm increments from 20 to 40 cm. Haiyaha was sectioned in 0.5-cm increments from 0 to 15 cm and thereafter in 1-cm increments. Sediment was stored in Whirl Pak® bags in the dark at 4°C until processed.

Water content was determined by weight loss during oven drying at 105°C. ^{210}Po was extracted and measured from an oven-dried aliquot of sediment (generally 0.5–1.0 g) by the method of Eakins and Morrison (1978); an internal ^{208}Po standard was added for efficiency calculations. This standard was

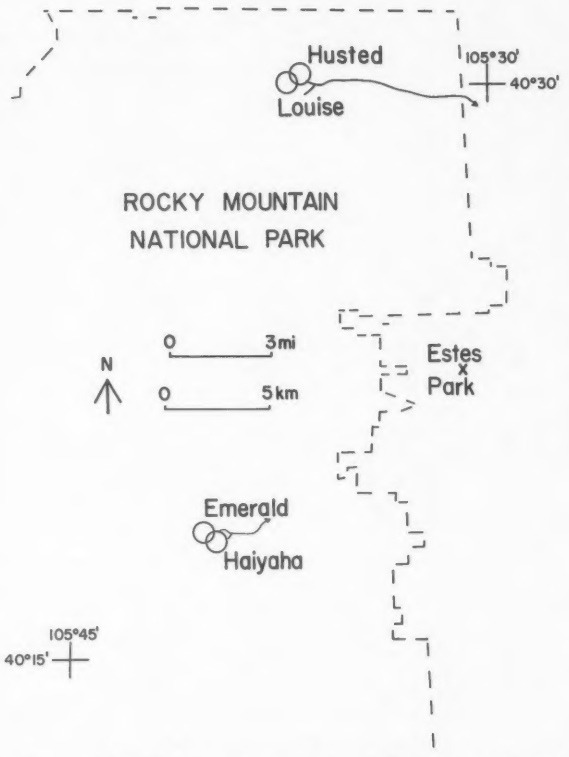

FIG. 1. Location of the study lakes. Estes Park is 45 km southwest of Fort Collins, Colorado.

obtained from the Amersham Scarle Co., Arlington Heights, IL. The ^{208}Po and ^{210}Po decays were counted, generally for 20 000 s, on a surface barrier alpha detector. From this, because of the time delay from sediment collection to measurement, secular equilibrium between ^{210}Po and ^{210}Pb was assumed; ^{210}Pb activity was calculated from ^{210}Po activity. Quality assurance procedures include remeasurement of sediment from previous studies (e.g. Davis et al. 1984), measurement of replicate samples, replicate measurement of the same samples, and interlaboratory comparisons.

Water samples from the four lakes were collected at approximately the same location as the sediment coring. Water samples were collected in late July 1983 using a Van Dorn sampler; subsamples were immediately transferred to BOD glass bottles and glass scintillation vials containing a fluor and shipped to Orono, ME, for ^{222}Rn analysis by liquid scintillation. We used

New England Nuclear Liquifluor. We counted the samples on July 29, 1983, and again on August 5, 1983, for 30 min. Radium standards from the Environmental Protection Agency (EPA), Las Vegas, NV, were used to prepare standard ^{222}Rn solutions. The samples in the BOD bottles were allowed to age and aliquots were recounted in late October 1983 to determine if the initial ^{222}Rn activity was supported by ^{226}Ra in solution.

^{222}Rn generation in sediment was evaluated by allowing ^{222}Rn to grow into equilibrium with ^{226}Ra from sediment samples in liquid scintillation vials (Wadach and Hess 1985). An aliquot of 1.0 g of oven-dried (105°C) sediment was placed in a liquid scintillation vial with 10 mL of water and allowed to become wet; then, 5 mL of New England Nuclear Liquifluor was added. The vial was sealed and stored for 1 mo. The ^{222}Rn, which had grown in, was counted for 10 h by liquid scintillation. An EPA ^{226}Ra standard was used for calibraton.

Results and Discussion

Our laboratory has analyzed approximately 50 lake sediment cores from New England, New York, Minnesota, and Scandinavia for ^{210}Pb. In New England, surface sediment ^{210}Pb activity ranges from 0.37 to 3.00 Beq/g dry sediment. In northern Sweden and southern and central Norway, values range from 0.67 to 3.33 Beq/g. Sediments from lakes in New York and Minnesota have similar values. Maximum integrated unsupported $^{210}Pb/cm^2$ was 1.30 and 0.56 Beq/cm^2 for New England and Scandinavia, respectively (Davis et al. 1984). In the New England and Scandinavia data sets, the surface sediment activity for ^{210}Pb was significantly correlated with water depth at the coring site. The study lakes reported herein fell within the morphometric parameters of those data (Davis et al. 1984).

Figure 2 displays the ^{210}Pb profiles for the four lakes. Lake Louise has a maximum measured ^{210}Pb activity that is an order of magnitude higher than in any lakes in the Davis et al. (1984) data. Lakes Husted and Emerald are also significantly higher in ^{210}Pb activity than the range of Davis et al.'s (1984) data for New England and Scandinavia. Furthermore, the integrated unsupported $^{210}Pb/cm^2$ is high except for Haiyaha: Louise, 11.0 Beq; Husted, 1.52 Beq; Haiyaha, 0.81 Beq; and Emerald, 2.41 Beq. These four lakes have no permanent surface inlets; their tendency to focus sediment (^{210}Pb) is normal as indicated by their morphology; precipitation (and associated scavenging of atmospheric ^{210}Pb) ranges from 1 to 2 m; a 10-fold increase in ^{210}Pb cannot be explained by atmospheric flux alone (Turekian et al. 1977). Based on the close proximity of the individual lakes of each pair, we suggest that each should receive equivalent areal loading of ^{210}Pb if wash-in of Pb from the drainage basin is unimportant. Sedimentation rates based on ^{210}Pb (grams per square centimetre per year) of major and minor constituents are typical for alphine lakes, based on our experience. We suggest that, at least for Louise, there was a high flux of ^{222}Rn to the lake via groundwater. The ^{222}Rn that did not escape to the atmosphere or flow out of the lake would decay "quantitatively" to ^{210}Pb within a few weeks and be scavenged by sedimenting particulates.

^{222}Rn is typically very low in surface water (<0.37 Beq/L) (see e.g. Imboden and Emerson 1978) although stream values may locally exceed those values due to the influx of groundwater (Breland and Fanning 1982; Rogers 1958). Groundwater may have values from 3.7 to 3.7 × 10^4 Beq/L, depending on the geological substrate (Brutsaert et al. 1981). Data in Table 2 indicate that Lake Louise water has high ^{222}Rn activity and is receiving ^{222}Rn-charged groundwater. The lake appears to have been well mixed except for the bottom water. The other three lake waters have much lower ^{222}Rn activities but values are higher than is typical for lakes. The ^{222}Rn is unsupported by ^{226}Ra in the water column as indicated by a lack of ^{222}Rn in samples held for 4 mo and then recounted (Table 2). Groundwater typically moves less than 10 m/d, and ^{222}Rn has a half life of 3.85 d. These facts imply that the relatively long-lived ^{226}Ra parent of ^{222}Rn is close to the lake, in the bedrock/soil system.

TABLE 2. ^{222}Rn activity (corrected for decay) for water samples from four lakes in Rocky Mountain National Park, Colorado (ND = not determined).

	Depth (m)	Collection July 1983 (Beq/L)	July 1983 samples remeasured on October 27, 1983 (Beq/L)
Lake Louise	1	82	<0.37
$\bar{z}$ = 2 m	2	98	<0.37
	3	106	<0.37
	4	104	<0.37
	5	109	<0.37
Lake Husted	1	6.92	<0.37
$\bar{z}$ = 3 m	2	3.96	<0.37
	3	2.48	<0.37
	4	1.00	<0.37
	5	6.00	<0.37
	6	0.52	<0.37
	7	7.55	<0.37
	8	7.07	<0.37
Emerald Lake	0.5	<0.37	ND
$\bar{z}$ = 8 m	7[a]	1.07	ND
	7[a]	1.89	ND
	13	6.14	ND
Lake Haiyaha	0.5	1.96	ND
$\bar{z}$ = 7 m	4.5[a]	2.37	ND
	4.5[a]	2.74	ND
	9.0	3.00	ND

[a]Replicates.

^{222}Rn can be lost from a lake in three ways: egress of lake water either as groundwater or surface water, degassing to the atmosphere (Emerson 1975), and decay to ^{210}Pb. We are unable to evaluate the first option rigorously. Degassing is suggested by the upper few metres of the profile in the Lake Louise water column. To estimate the radon that is available to these lakes due to outdoor air, we refer to the works of Gesell (1983), Wilkening (1980), and Larson and Bressan (1980); these authors state that the radon content in outside air is approximately 9 × 10^{-3} Beq/L. We have no air data for the study area. The equilibrium partitioning between air and water over the temperature range 0–20°C is 1:1. Therefore, the expected radon concentration in the water, if it is in equilibrium with typical ^{222}Rn values in air, is about 9 × 10^{-3} Beq/L. Therefore, degassing should occur from the ^{222}Rn-rich water and the atmosphere cannot be the source of excessive ^{222}Rn.

An alternative possible source for the excess ^{222}Rn is the ^{226}Ra in the sediment. Table 3 shows data relating to ^{226}Ra content of the lake sediment. As one estimate of this contribution, we evaluated the supported ^{210}Pb in the sediment (Fig. 2). We also used ^{222}Rn emanation by the sediment to estimate

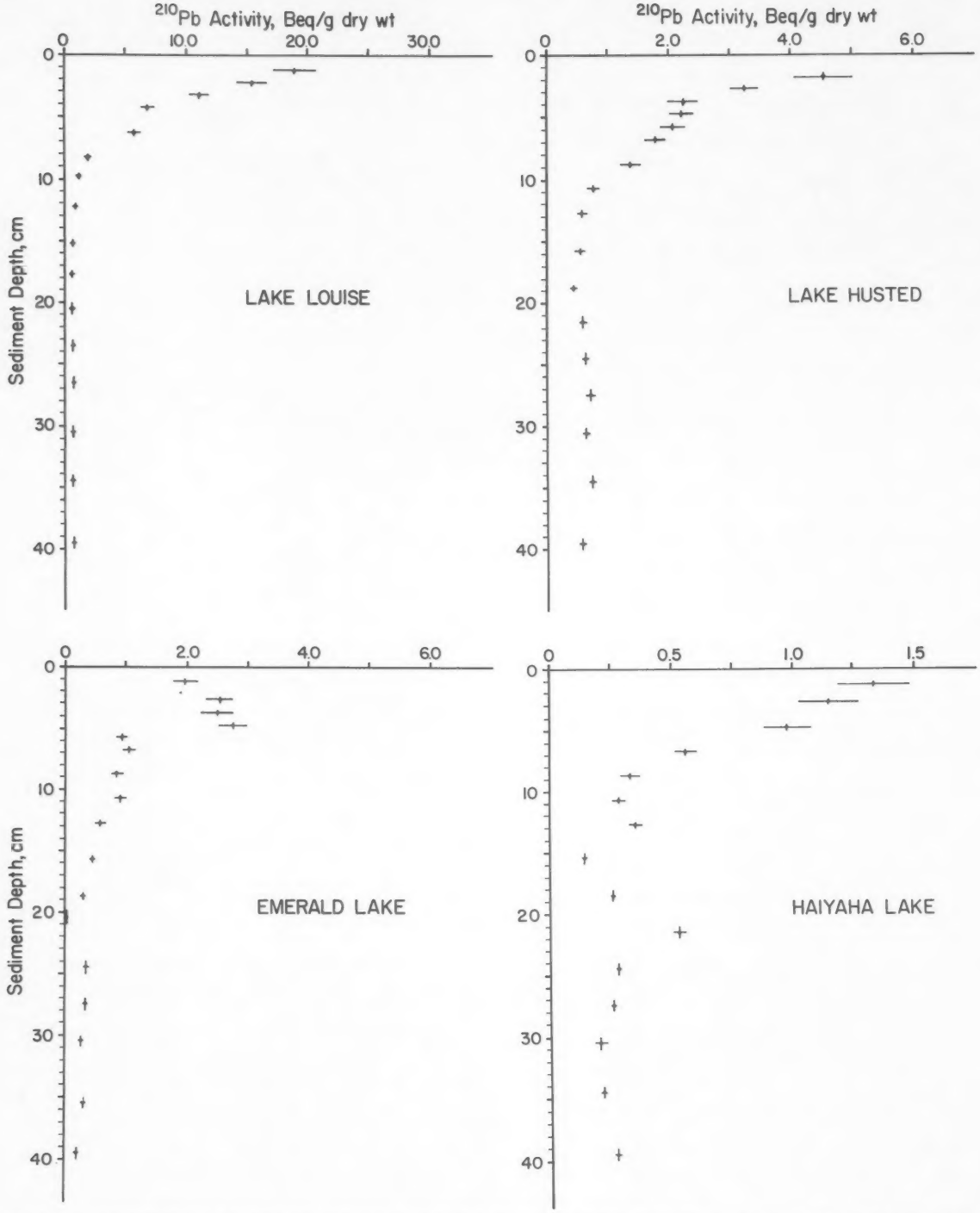

FIG 2. ^{210}Pb activity (Beq/g dry wt.) in lake sediment versus depth for the four study lakes, Rocky Mountain National Park, Colorado. Horizontal bars represent 1 SD (counting error). Vertical bars indicate the sample interval.

TABLE 3. Radium activity of selected samples of lake sediment measured by ^{222}Rn emanation for three lakes in Rocky Mountain National Park, Colorado.

	Sediment depth (cm)	^{226}Ra from ^{222}Rn emanation (Beq/g)	^{226}Ra from ^{210}Pb profile, assuming equilibrium (Beq/g)
Lake Louise	4.5–5.0	0.24	— (contains unsupported ^{210}Pb)
	22–23	0.20	0.70 (interpolated)
Emerald Lake	4.5–5.0	0.11	— (contains unsupported ^{210}Pb)
	30–31	0.10	0.33 (interpolated)
Lake Husted	2.5–3.0	0.01	— (contains unsupported ^{210}Pb)
	21–22	0.29	0.59 (measured)
	32–33	0.09	0.67 (interpolated)

TABLE 4. ^{222}Rn generation by the sediment from four lakes in Rocky Mountain National Park, Colorado.

	$\bar{z}$ (m)	Supported Pb (Beq/g dry wt)	g dry sediment (1)/ g wet sediment (10) (Beq/g wet wt)	Top 3 cm wet sediment × 1 cm^2 (Beq)	Beq/L released to lake water (Beq/L)
Lake Louise	2	0.74	0.07	0.22	1.11
Lake Husted	3	0.93	0.09	0.28	0.93
Lake Haiyaha	7	0.26	0.03	0.08	0.11
Emerald Lake	8	0.44	0.04	0.13	0.17

^{226}Ra; this technique underestimates the total ^{226}Ra because of the inability of ^{222}Rn to escape from the interior of mineral grains. The ^{210}Pb determination is based on an extraction of the highly volatile ^{210}Po at 550°C and probably extracts a greater percentage of the ^{226}Ra daughter than ^{222}Rn emanation does at 20°C. Consistent with this, ^{226}Ra estimated from ^{222}Rn emanation is less than ^{226}Ra estimated from ^{210}Po.

Based on ^{210}Po measurements and assuming secular equilibrium between ^{226}Ra and all the daughters to ^{210}Po, we estimated ^{222}Rn generation at 0.74 Beq/g dry weight (a maximum for Louise), 0.93 Beq/g dry weight (Husted), 0.26 Beq/g dry weight (Haiyaha), and 0.44 Beq/g dry weight (Emerald) (Table 4). Based on an approximate water content of 90% in the upper 3 cm of sediment, we have calculated the wet sediment ^{226}Ra activity (Table 4, column 3). We assume that only 3 cm of sediment can contribute significant ^{222}Rn to the water column, considering the diffusion constant for ^{222}Rn and its decay. Table 4, column 4, lists the total ^{222}Rn generated in the upper 3 cm of sediment, all of which is assumed to diffuse upward to the water column and mix instantaneously. The last column is the resulting ^{222}Rn activity of the water column resulting from the sediment input (0.1 L·m lake depth^{-1}·cm^{-2}). Clearly the existing ^{222}Rn activities in lake water (Table 2) are too high to be explained by generation of ^{222}Rn in the sediment and release to the water column. The remaining alternative for the source of the high ^{210}Pb activity is the water column.

We have calculated the ^{210}Pb activity in Lake Louise sediment due to the water column ^{210}Pb production. Our calculations assume that the ^{222}Rn activity in the water column is constant throughout the year. We have only one time of sampling. We assume that any ^{210}Pb produced in the water column is sedimented with 100% efficiency, at the spot beneath the water column (i.e. there is no secondary sediment focusing). The lake has a flushing rate of less than 1 mo (see Table 1). Therefore, the ^{222}Rn in the water column cannot be in secular equilibrium with the ^{210}Pb. The 6-m water column at the coring site has a ^{222}Rn activity of 98 Beq/L (Table 2) and produces 1.9×10^9 atoms ^{210}Pb/yr. This leads to a ^{210}Pb activity of 2.1 Beq/cm^2 added to the annual increment of sediment dry mass. The latter is about 0.01 g·cm^{-2}·yr^{-1}.

Thus the sediment, when deposited, would have an initial activity of unsupported ^{210}Pb of 210 Beq/g. This is more than adequate to explain the high activities observed in Lake Louise sediment.

With comparable assumptions as above, atmospheric deposition of 3.7×10^{-3} Beq $^{210}Pb/cm^2$ (corresponding to 3.7×10^{-2} Beq/L and 1 m of precipitation) would yield 0.37 Beq/g. This value is slightly less than the average values cited by Davis et al. (1984) for surface sediments from a large set of lake cores. We suggest that surface sediment activities of >2 Beq/g ^{210}Pb are probably explained only in terms of considerable *element-selective* (Pb) focusing, very low sedimentation rates, or the influence of ^{222}Rn-rich groundwater entering the lake. Of the four lakes reported on herein, only Haiyaha appears to have a ^{210}Pb profile that is relatively uninfluenced by groundwater.

We emphasize that so long as the flux of ^{222}Rn to the lake via groundwater is relatively constant and in-lake processing is steady state, ^{210}Pb dating may still be valid.

The high ambient ^{222}Rn in the water column of Lake Louise, and to a lesser extent Emerald and Husted, is maintained nearly entirely by groundwater influx. This high ^{222}Rn activity may be responsible for an abnormally high flux of ^{210}Pb to the sediment surface yielding a high integrated unsupported ^{210}Pb for the sediments. Under these conditions if sedimentation rates are typical, which they appear to be, the result should be high ^{210}Pb activity in the surface sediment.

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References

BRELAND, J. A., AND K. A. FANNING. 1982. ^{226}Ra and ^{222}Rn in rivers and estuaries of western Florida. EOS, Trans. Am. Geophys. Union 63: 56.

BRUTSAERT, W. F., S. A. NORTON, C. T. HESS, AND J. S. WILLIAMS. 1981. Geologic and hydrologic factors controlling radon-222 in groundwater in Maine. Ground Water 19: 407–417.

COLE, J. P. 1977. Geology of east-central Rocky Mountain National Park and vicinity, with emphasis on the emplacement of the Precambrian Silver Plume Granite, in the Longs Peak – St. Vrain bathilith. Ph.D. thesis, University of Colorado, Boulder, CO. 393 p.

DAVIS, R. B., AND R. W. DOYLE. 1969. A piston corer for upper sediment in lakes. Limnol. Oceanogr. 14: 643–648.

DAVIS, R. B., C. T. HESS, S. A. NORTON, D. W. HANSON, K. D. HOAGLAND, AND D. S. ANDERSON. 1984. ^{137}Cs and ^{210}Pb dating of sediments from soft-water lakes in New England and Scandinavia, a failure of ^{137}Cs dating. Chem. Geol. 44: 151–185.

DILLON, P. J., AND P. J. SMITH. 1983. Lead-210 fluxes in acidified lakes (Abstr. of Proceed.). Soc. Int. Limnol. Lyon 81.

EAKINS, J. D., AND R. T. MORRISON. 1978. A new procedure for the determination of ^{210}Pb in lake and marine sediments. Int. Appl. Rad. Iso. 29: 531–536.

EMERSON, S. 1975. Gas exchange rates in small Canadian Shield lakes. Limnol. Oceanogr. 20: 754–761.

EVANS, R. D., AND F. H. RIGLER. 1983. A test of lead-210 dating for the measurement of whole lake soft sediment accumulations. Can. J. Fish. Aquat. Sci. 40: 506–515.

GESELL, T. F. 1983. Background atmospheric ^{222}Rn concentrations outdoors and indoors. A review. Health Phys. 45: 289–302.

HANSON, D. W., S. A. NORTON, AND J. S. WILLIAMS. 1982. Modern and paleolimnological evidence for accelerated leaching and metal accumulation in soils in New England, caused by atmospheric deposition. Water Air Soil Pollut. 18: 227–239.

IMBODEN, D. M., AND S. EMERSON. 1978. Natural radon and phosphorus as limnologic tracers: horizontal and vertical eddy diffusion in Greifensee. Limnol. Oceanogr. 23: 77–90.

IMBODEN, D. M., AND M. STILLER. 1982. The influence of radon diffusion on the ^{210}Pb distribution in sediments. J. Geophys. Res. 87: 557–565.

JOHNSON, R. B., AND D. J. HERZOG. 1983. Summary of geologic factors that may influence the sensitivity of selected watersheds in Rocky Mountain National Park, Colorado to atmospheric deposition. Rep. 83-6: 23 p. (Available from Water Resources Field Support Laboratory, Fort Collins, CO.)

JOHNSTON, S. E., S. A. NORTON, C. T. HESS, R. B. DAVIS, AND R. S. ANDERSON. 1982. Chronology of atmospheric deposition of acids and metals in New England, based on the record in lake sediments, p. 177–187. *In* L. H. Keith [ed.] Energy and environmental chemistry, acid rain, Vol. 2. Ann Arbor Science Publishers, Ann Arbor, MI.

LARSON, R. E., AND D. J. BRESSAN. 1980. Radon-222 as an indicator of continental air masses and air mass boundaries over ocean areas, p. 308–326. *In* T. F. Gesell and W. M. Lowder [ed.] Natural radiation environment III. Vol. 1. Tech. Inf. Center/U.S.D.O.E., Springfield, VA.

NESSE, W. D. 1972. Geology and metamorphic petrology of the Pingree Park Area, northeast Front Range, Colorado. Ph.D. thesis, University of Colorado, Boulder, CO. 265 p.

NORTON, S. A. 1983. Sediment chemistry of four lakes in Rocky Mountain National Park, CO. Final Report. 57 p. (Available from Water Resources Field Support Laboratory, Fort Collins, CO.)

NORTON, S. A., AND J. S. KAHL. 1983. Deposition of atmospheric Pb and Zn in New England lake sediment. Proc. Int. Conf. Heavy Metal Environ., Heidelberg. CEP Consultants Ltd. Vol. 1. Edinburgh, UK.

ROGERS, A. S. 1958. Physical behavior and geologic control of radon in mountain streams. U.S. Geol. Surv. Bull. 1052-E: 187–211.

TUREKIAN, K. K., Y. NOZAKI, AND L. K. BENNINGER. 1977. Geochemistry of atmospheric radon and radon products. Annu. Rev. Earth Planet. Sci. 5: 227–255.

WADACH, J. B., AND C. T. HESS. 1985. Radon concentration measurements in soil using liquid scintillation and track etch. Health Phys. 48. (In press)

WILKENING, M. 1980. Radon transport processes below the Earth's surface, p. 90–104. *In* T. F. Gesell and W. M. Lowder [ed.] Natural radiation environment III. Vol.1, Tech. Inf. Center/U.S.D.O.E., Springfield, VA.

An Improved Photo-Electric Fish Egg Counter

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Tanasichuk, R. W., C. Armstrong, and D. M. Ware. 1985. An improved photo-electric fish egg counter. Can. J. Fish. Aquat. Sci. 42: 1255–1258.

We describe an improved design and operating procedure for a photo-electric fish egg counter. The design was simplified so that the apparatus can function as a flow-through system using tap water. The procedure for operating the counter was changed so that coincidence counting can be corrected for. Triplicate fecundity determinations, made gravimetrically for 10 randomly selected Pacific herring (*Clupea harengus pallasi*), were repeated using our method to compare the accuracy and precision of estimates made with both procedures. Mean fecundity estimates did not differ significantly between methods. The precision of estimates made using the counter was at least sixfold greater, and single determinations were accurate to within 5% of the true mean value with 95% probability. Our procedure is also less tedious and about 70% faster than the gravimetric method. The counter, unmodified, should be capable of counting eggs from 25 marine and 6 freshwater fish species. We believe that it can be easily adapted to count eggs that differ substantially in size from those of Pacific herring.

Les auteurs décrivent un modèle et un mode de fonctionnement améliorés d'un compteur photo-électrique d'œufs du poisson. La conception a été simplifiée afin que le dispositif puisse fonctionner comme un système à débit continu avec de l'eau du robinet. On a modifié le mode de fonctionnement de manière que le dénombrement coïncident puisse être éliminé. Des déterminations en triple, par gravimétrie, de dix harengs du Pacifique (*Clupea harengus pallasi*) choisis au hasard ont été répétées selon notre méthode pour une comparaison de l'exactitude et de la précision des estimations effectuées à l'aide des deux méthodes : la fécondité moyenne estimative n'a pas varié de façon significative. La précision des estimations obtenues au compteur était au moins six fois plus élevée et les déterminations individuelles se situaient dans les 5 % de la vraie valeur moyenne avec une probabilité de 95 %. La méthode présentée est aussi moins fastidieuse et environ 70 % plus rapide que la méthode gravimétrique. Le compteur dans son état actuel devrait pouvoir dénombrer les œufs de 25 espèces de poissons marins et de 6 espèces de poissons dulçaquicoles. Selon les auteurs, il pourrait être facilement adapté pour le comptage des œufs dont la taille varie nettement par rapport à celle des œufs du hareng du Pacifique.

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Fish fecundity can be estimated from total egg counts or from volumetric or gravimetric subsamples. Eggs can be counted visually when numbers are low; otherwise, egg counters such as those described by Parrish et al. (1960) and Boyar and Clifford (1967) are used. Bagenal and Braum (1978) described how fecundity is estimated using volumetric or gravimetric subsamples. Fecundity determinations tend to be made using gravimetric subsamples, presumably because it is the easiest method to implement.

To date, the fecundity of Pacific herring (*Clupea harengus pallasi*) in British Columbia has been estimated using the gravimetric method (see Hourston et al. 1981). This procedure is tedious, relatively slow (an average of only three fecundity estimates can be made per hour), and subject to substantial counting and weighing errors. These limitations discouraged us from using the method for a coastwide fecundity monitoring program.

Our objective was to determine if an improved version of the photo-electric counter described by Parrish et al. (1960) could provide fecundity estimates more efficiently yet as accurately as the gravimetric method does.

Methods

The design of the fish egg counter developed by Parrish et al. (1960) and our modification of it are shown in Fig. 1 and 2, respectively. Both counters operate by the same principle: eggs pass through the apparatus in a stream of water and break a light beam that shines across the counting chamber; interruptions of the beam are tallied electronically. We have eliminated the constant head tank. Water enters our counter, via tygon tubing, through a port in the bottom of the reservoir. The minimum internal diameter of the counting chamber was increased to 2.5 mm to maximize the water flow through the counter while minimizing the probability of eggs moving through the counting chamber abreast. Counted eggs are collected in a 250-mL Nalgene jar with a 300-μm plastic mesh bottom. This jar is suspended in a larger vessel which collects all water that flowed through the apparatus while eggs were counted. A sensitivity adjustment was added to eliminate counting any inclusions (i.e. air bubbles, egg membrane fragments).

Results of experiments designed to determine the accuracy of counts made with our apparatus indicated that the modified

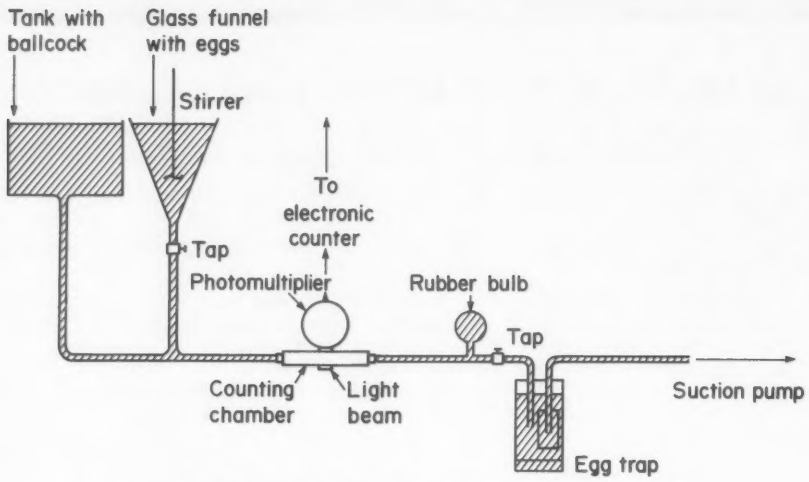

FIG. 1. Prototype photo-electric fish egg counter (Parrish et al. 1960).

counter consistently underestimated the number of eggs in samples of known sizes. Replicate counts ($n = 5$) of samples containing 250, 500, or 1000 eggs were an average of 1.3, 2.0, and 5.2% lower, respectively, than the known number of eggs. Subsequent counts of a sample containing 3000 eggs, made at various egg concentrations, indicated that the error was a function of egg concentration. We assume that the error results from coincidence counting.

To correct for this error, we developed a calibration curve that relates average egg concentration (eggs per litre) to the number of eggs counted per litre. The curve was generated by making five replicate counts of a series of standards which consisted of samples containing 1000, 2000, 3000, 4000, 5000, or 6000 eggs. Samples were counted over about 1 min. This meant that egg concentrations ranged between approximately 1100 and 6600 eggs$\cdot$L^{-1} because the flow rate through the counter averages 900 mL$\cdot$min^{-1}. Standards larger than 6000 eggs were not prepared because past experience indicated that the counting chamber plugs frequently at egg concentrations greater than about 7000$\cdot$L^{-1}.

The operating procedure for the counter was modified so that we could estimate mean counts per litre and consequently correct the error due to coincidence counting. Parrish et al. (1960) reported that their apparatus is operated by placing eggs in water in a large glass funnel, suspending them with a stirrer, and then allowing the eggs to pass through the counting chamber by opening a tap at the base of the funnel. To use our counter, aliquots containing similar numbers of eggs are added at regular intervals and the water volume flowing through the apparatus during the determination is measured. Average egg concentration is calculated from the calibration curve using the estimated counts per litre; egg concentration is then multiplied by water volume to yield the estimated number of eggs.

The accuracy and precision of fecundity estimates made with the gravimetric method were compared with those for estimates obtained by our procedure. We used the modified counter to repeat triplicate fecundity determinations made gravimetrically for 10 randomly selected fish. Estimates were made using the counter at a relatively low (~2500 counts$\cdot$L^{-1}) and then at a relatively high (~5000 counts$\cdot$L^{-1}) counting rate to examine variations in accuracy and precision over the anticipated operating range.

Results

The calibration curve we developed to describe the relationship between known egg concentration and egg counts per litre is presented in Fig. 3. The coefficient of determination ($r^2 = 0.999$) indicates that virtually all of the variation in counts per litre is accounted for by its linear relationship with egg concentration.

Fecundity estimates obtained using the gravimetric method and the counter operating at both counting ranges are presented in Table 1. Results of a two-way ANOVA, completed after applying the square root transformation to total counts recommended by Sokal and Rohlf (1981), indicated that differences between ovary samples ($p = 0.0001$), but not among the three methods ($p = 0.12$), accounted for the variation between mean fecundity estimates. After using the angular transformation for percentage data recommended by Sokal and Rohlf (1981), we found that the mean estimates obtained using the gravimetric method were 2.1 ± 7.78% ($\bar{x}$ ± SD) higher than those made with our method. Results of the G-test (Sokal and Rohlf 1981) indicated that the signs of percent difference of estimates between methods varied randomly ($G_{adj} = 1.55$). Thus, the counter has no tendency to overestimate or underestimate fecundity relative to the gravimetric method.

Estimates made using our method are extremely precise. Coefficients of variation, calculated after applying the angular transformation recommended by Sokal and Rohlf (1981), were 0.41 ± 0.11% and 0.5 ± 0.09% ($\bar{x}$ ± SD) for determinations made operating the counter at approximately 2500 and 5000 counts$\cdot$L^{-1}, respectively. Estimates made using our procedure are precise enough that single determinations provide acceptable accuracy, i.e. estimates to within 5% of the true mean value with 95% probability. Snedecor and Cochran (1967) provided an equation for calculating the sample size required to estimate the true mean value with a prescribed precision at a specified probability level:

$$n = (as)^2 \cdot (L^2)^{-1}$$

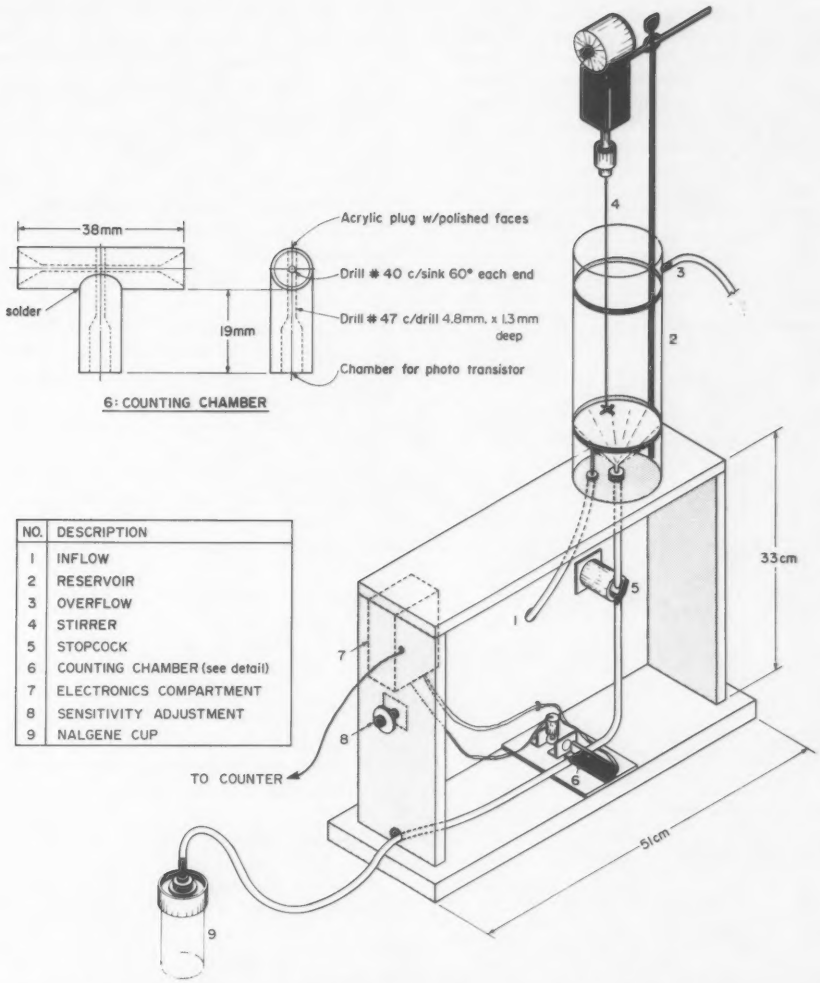

FIG. 2. The modified counter. Plans, wiring diagrams, and a description of the operating procedure can be obtained by writing to the senior author.

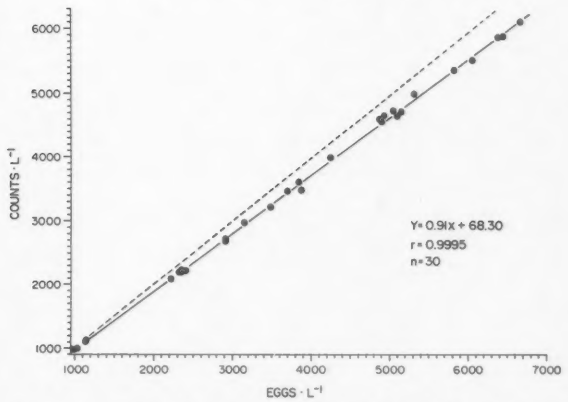

FIG. 3. Regression of counts·L^{-1} against eggs·L^{-1}. Broken line indicates perfect agreement between variables.

where n = sample size, a = the value of t from the two-tailed t-table for infinite df at 5 or 1%, s = the standard deviation of the sample, and L = allowable error in the sample mean. We calculated that fecundity estimates accurate to within 5% of the true fecundity with 95% probability would require sample sizes of 0.08 ± 0.160 ($\bar{x}$ ± SD) and 0.09 ± 0.180 ($\bar{x}$ ± SD) when the counter is operated at the low and high counting rates, respectively.

Discussion

Our method enables fecundity estimates for Pacific herring to be made as accurately, more precisely, faster, and with less tedium than when using the gravimetric method. We found no significant differences among fecundities determined gravimetrically or with the counter operated at low or high counting rates. Each mean fecundity estimated gravimetrically resulted from a series of replicate determinations, with a replicate

TABLE 1. Comparison of fecundity estimates made gravimetrically and with the modified egg counter. Estimates made using the gravimetric method are based on a variable number of determinations because of conditions regarding agreement between replicates before one is accepted; therefore, statistics of dispersion (e.g. standard deviation) are underestimated and consequently not given. Triplicate estimates are presented in the order of method used: gravimentric, the counter operated at approximately 2500 counts·L^{-1}, and the counter operated at about 5000 counts·L^{-1}. Percent difference is the mean gravimetric estimate relative to the mean counter estimate.

Ovary No.	Mean	SD	CV (%)	Percent difference
1	36 392	—	—	+12.8
	32 131	34	0.1	
	32 377	38	0.1	
2	24 994	—	—	−0.2
	24 966	159	0.6	
	25 119	151	0.6	
3	58 549	—	—	+15.3
	50 524	927	1.8	
	51 014	368	0.7	
4	39 836	—	—	+2.2
	38 761	125	0.3	
	39 157	116	0.3	
5	23 121	—	—	+14.1
	20 168	138	0.7	
	20 252	131	0.6	
6	42 022	—	—	+10.8
	37 932	173	0.5	
	37 937	102	0.3	
7	20 857	—	—	+9.6
	19 315	6	0.03	
	18 757	371	2.0	
8	46 253	—	—	+3.4
	44 560	213	0.5	
	44 905	116	0.3	
9	20 119	—	—	−11.4
	22 774	111	0.5	
	22 642	153	0.7	
10	28 833	—	—	−8.9
	31 738	29	0.1	
	31 590	139	0.4	

accepted if it was within 5% of other replicates. Consequently, the standard deviation of each mean fecundity is underestimated. Despite this, the mean coefficients of variation for the estimates made using our procedure are still sixfold less than that ($2.7 \pm 0.34\%$, $\bar{x} \pm$ SD) for the fecundities determined gravimetrically. Based on an average fecundity of 25 000 eggs, we estimated that five herring fecundity determinations could be made per hour using our procedure in contrast with three obtained gravimetrically. Excluding the start-up procedure for the counter, determining fecundity with our method consists only of adding aliquots of eggs at 1-min intervals, measuring how much water flowed through the counter during the determination, and correcting for coincidence counting. The gravimetric method, however, requires that all eggs in an ovary subsample be counted visually after it is damp-dried and then weighed; a minimum of two determinations must be made.

The procedure outlined should provide more accurate fecundity estimates than the method described by Parrish et al. (1960) because it includes a correction for coincidence counting. Parrish et al. (1960) did not consider coincidence counting presumably because they assumed that small (500–800 eggs) and large (19 000 – 43 000 eggs) samples were counted with the same accuracy. We have shown that, when eggs pass through our apparatus at rates comparable with that recommended by Parrish et al. (1960) (approximately 6000 eggs·min^{-1}), coincidence counting must be corrected for to provide accurate fecundity estimates.

The modified photo-electric counter should be useful for estimating the fecundity of other fish species and for counting sperical particles in general. The apparatus is designed to count eggs ranging between 1.0 and 1.5 mm in diameter, i.e. a volume between 0.52 and 1.77 mm^3. Bagenal (1971) presented egg volumes for 46 marine and 27 freshwater fish species. Based on those data, our apparatus, unmodified, would be able to count eggs from 25 marine and 6 freshwater species. Our apparatus should be capable of counting eggs that differ substantially in size from those of Pacific herring. However, the bore of the counting chamber may have to be changed to preserve the optimum between maximizing flow rate (and therefore counting rate) and minimizing the incidence of coincidence counting. A calibration curve would also have to be developed.

Acknowledgments

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References

BAGENAL, T. B. 1971. The interrelationship of the size of fish eggs, the date of spawning and production cycle. J. Fish. Biol. 3: 207–219.

BAGENAL, T. B., AND E. BRAUM. 1978. Eggs and early life history, p. 165–201. *In* T. B. Bagenal [ed.] Methods for assessment of fish production in fresh waters. 3rd ed. Blackwell Scientific Publications, London.

BOYAR, H. C., AND R. A. CLIFFORD. 1967. An automatic device for counting dry fish eggs. Trans. Am. Fish. Soc. 96: 361–363.

HOURSTON, A. S., V. HAIST, AND R. D. HUMPHREYS. 1981. Regional and temporal variation in the fecundity of Pacific herring in British Columbia waters. Can. Tech. Rep. Fish. Aquat. Sci. 1009: 31 p.

PARRISH, B. B., I. G. BAXTER, AND M. J. D. MOWAT. 1960. An automatic fish egg counter. Nature (Lond.) 185: 777.

SNEDECOR, G. W., AND W. G. COCHRAN. 1967. Statistical methods. 6th ed. Iowa State University Press, Ames, IA. 593 p.

SOKAL, R. R., AND F. J. ROHLF. 1981. Biometry. 2nd ed. W. H. Freeman and Co., San Francisco, CA. 859 p.

Aggregative Response of Common Mergansers (*Mergus merganser*): Predicting Flock Size and Abundance on Vancouver Island Salmon Streams

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Wood, C. C. 1985. Aggregative response of common mergansers (*Mergus merganser*): predicting flock size and abundance on Vancouver Island salmon streams. Can. J. Fish. Aquat. Sci. 42: 1259–1271.

The abundance of common mergansers (*Mergus merganser*) was monitored on four salmon-producing streams on the east coast of Vancouver Island, B.C., during the spring and summer of 1980–83. Overall, merganser abundance declined steadily from March through June but increased following recruitment of juvenile birds. Mergansers congregated on streams where juvenile salmonid migrations were enhanced by hatcheries or spawning channels. Movement between streams was evident from inverse trends in abundance on three adjacent streams differing in juvenile salmonid production. At least 8 of 12 mergansers resighted after being marked and released on the Big Qualicum River visited other nearby streams. Flock-size distributions predicted by an equilibrium arrival–departure model were consistent with those observed during May–mid-June, but not those during late June. Social interactions and reduced flight activity appeared to influence dispersion to a greater extent during late June to August so that assumptions of the model were violated. A similar model was used to predict aggregation patterns on hatchery streams from observed relationships between fish availability and frequencies of arrival and departure. The aggregation model provides a parsimonious explanation of merganser aggregation patterns during March–June and is a potentially useful tool for designing fish release schedules to minimize predation by mergansers.

Au cours du printemps et de l'été de 1980 à 1983, on a surveillé le nombre de grands becs-scies (*Mergus merganser*) fréquentant quatre ruisseaux à saumons de la côte est de l'île de Vancouver (C.-B.). En général, le nombre d'oiseaux a baissé régulièrement de mars à juin mais a augmenté suite à un apport de juvéniles. Les becs-scies se sont regroupés dans les cours d'eau où le nombre de salmonidés juvéniles migrateurs était augmenté par l'exploitation de piscifactures ou de frayères artificielles. Des tendances inverses de l'abondance dans trois ruisseaux voisins à production variable de saumons juvéniles sont la preuve de déplacements des oiseaux d'un cours d'eau à l'autre. Au moins 8 des 12 becs-scies observés à nouveau après le marquage et le lâcher dans la rivière Big Qualicum ont visité d'autres cours d'eau voisins. La répartition des volées selon la taille, calculée à l'aide d'un modèle d'équilibre des arrivées et des départs, était compatible avec la répartition observée de mai à la mi-juin mais non avec celle signalée à la fin juin. Les interactions sociales et l'activité de vol diminuée semblaient influer plus fortement sur la dispersion de la fin juin à août; les hypothèses du modèle n'ont donc pas été respectées. On a utilisé un modèle semblable pour prédire les schèmes de regroupement sur les cours d'eau d'écloserie à partir des relations entre la disponibilité des poissons et la fréquence d'arrivée et de départ. Ce modèle fournit une explication restreinte des schèmes de regroupement des becs-scies de mars à juin; il présente toutefois un potentiel pour la détermination des calendriers de lâcher des poissons afin de minimiser l'action prédatrice des becs-scie.

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The search behaviour of individual predators is well adapted to exploit local concentrations of their prey (e.g. Smith and Sweatman 1974). Consequently, predators that forage independently of each other should accumulate at favourable feeding sites. Ample evidence supports this expectation (e.g. Rusch et al. 1972; Readshaw 1973; O'Connor and Brown 1977; Phelan and Robertson 1978; Bell 1980; Village 1982) and the phenomenon is usually referred to as an "aggregative response" (Readshaw 1973). For species that feed on valuable resources, it is useful to know the factors that govern the aggregative response and, thus, the intensity of predation in specified areas.

My objective was to identify conditions that attract concentrations of common mergansers (*Mergus merganser*) in salmonid-rearing areas. Mergansers pose a serious threat to salmonid fisheries, but only when they are abundant on the freshwater reaches of salmonid-producing streams or lakes; elsewhere, mergansers do little damage (Salyer and Lagler 1940; White 1957; Wood 1984). By understanding how these feeding aggregations arise, efforts could be made to curtail their size and duration and, hence, to reduce depredations by mergansers. My aim was to demonstrate that the size of aggregations on a hatchery-enhanced stream depends on the size of the merganser population within ranging distance (the "effective" population)

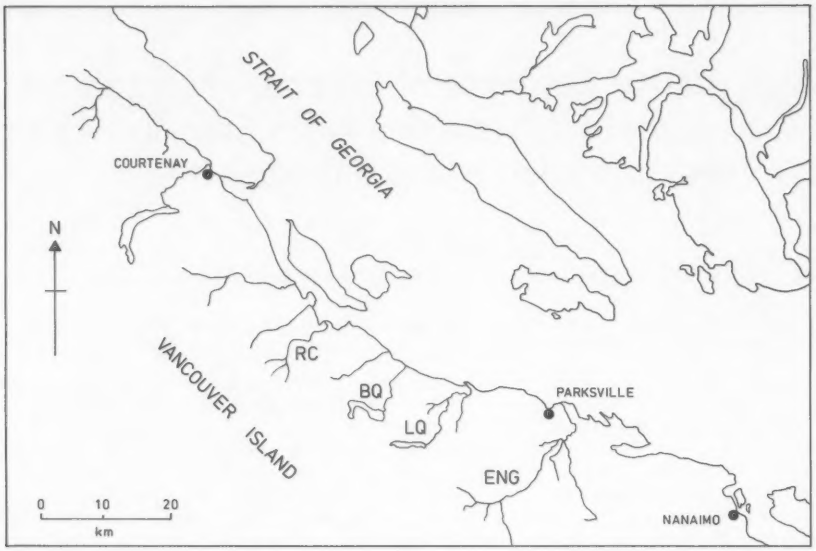

FIG. 1. Censused areas. RC, Rosewall Creek; BQ, Big Qualicum River; LQ, Little Qualicum River; ENG, Englishman River.

and on the pattern of change in prey availability within the stream.

Aggregation Model

The effective population size depends both on the number of birds in the vicinity and on the distance they venture each day in search of food. As with most waterfowl, merganser populations undergo dramatic seasonal changes in abundance and sex composition associated with nonfeeding behaviour (e.g. moults, migration, and reproduction). Consequently, the effective population size will depend on the season, and perhaps on daily and tidal cycles. Typical ranging patterns of mergansers were inferred from flight and foraging activity and from the movements of marked individuals. Pooled census data for the study area were examined to reveal seasonal trends in merganser abundance and flock composition unrelated to salmonid density. Deviations from these expected trends were then examined for each stream in relation to juvenile salmonid migrations.

The simplest approach to modeling the aggregative response is to assume that each merganser forages independently on the basis of its own information and decision rules (Wood 1985). This is the essence of the equilibrium flock-size (EFS) model proposed by Krebs (1974) whereby birds accumulate at a favourable site until the number joining the flock is balanced by the number leaving. Under this hypothesis, the decision to join or desert a flock is made independently by individual birds or cohesive groups of individuals such as breeding pairs. However, arrival and departure decisions need not be independent of flock size or other information conveyed by the flock; the effects of social interactions or "local enhancement" (Thorpe 1963) are accommodated easily in the following general form of the model:

$$(1)\quad \frac{dF}{dt} = a(F)(N - F) - d(F, P)F$$

where F represents flock size, N is the effective population, P is the profitability at the site, and a and d are functions defining the probabilities of arrival and departure, respectively.

The aggregative response to a potential feeding site will depend on the relative profitability (i.e. attractiveness) of alternative sites. However, it is often possible to apply the EFS model in situations where the profitability of nearby sites changes little in comparison with the site of interest. For example, the EFS model has been applied with considerable success to feeding flocks of great blue herons (*Ardea herodias*) (Krebs 1974) and house sparrows (*Passer domesticus*) (Barnard 1980); stochastic versions of the model were also evaluated by Caraco (1980) for yellow-eyed junco (*Junco phaeonotus*) flocks.

Mergansers spend relatively little time foraging (Wood and Hand 1985) so the EFS model could not be applied strictly to foraging flocks. Instead, I assumed that mergansers assessed profitability while foraging and that the probability of departure depended on recent foraging success regardless of whether the birds were still foraging (Wood 1985). The EFS model was evaluated on the basis of qualitative predictions about flock-size distributions. I also adapted the EFS model to predict the size of aggregations on a larger spatial scale (i.e. collections of merganser flocks). Predicted aggregation patterns were compared with observed trends on two streams with hatchery-enhanced salmonid migrations.

Methods

Study Area and Census Procedures

Four neighbouring salmon streams on the east coast of Vancouver Island were censused regularly from March to September during 1980–83. These included, from largest to smallest, Englishman River (ENG), Little Qualicum River (LQ), Big Qualicum River (BQ), and Rosewall Creek (RC); the location and relevant statistics of each are presented in Fig. 1 and Table 1. Salmon hatcheries are situated on the latter three. Scheduled fish releases from hatcheries provided an opportunity to monitor the responses of mergansers to repeated, large-scale

TABLE 1. Summary of statistics pertaining to juvenile salmonid production of rivers censused, 1980–82.

River system	Drainage area (km^2)	Length accessible to salmon (km)[a]	Area of intertidal within 1 km of outlet (km^2)	Year	Wild spawners (thousands)[b]	Juvenile migrations (JSI in millions)		
						All fry	Smolt and fry: March–May	Smolt and fry: Total
Englishman	283	16.0	0.8	1981	2.5	3.0	3.8	4.0
				1982			3.8	4.0
Little Qualicum[c]	246	11.5	1.1	1980	6.6	20.0	20.4	27.0
				1981		9.0	9.6	17.0
Big Qualicum[c]	150[d]	10.4	0.6	1980	6.5	67.0	74.9	93.0
				1981		60.0	87.3	95.0
				1982		44.0	55.6	76.0
Rosewall Creek[c]	45	3.8	1.5	1980	0.4	0.8	1.8	2.5
				1981			0.9	1.6

[a]Marshall et al. 1977.
[b]Index of wild smolt migration (30 yr avg. for coho, chinook, and steelhead spawners, Marshall et al. 1977).
[c]Streams with hatchery.
[d]Drainage area below dam is 44 km^2.

perturbations of juvenile salmonid density. Reliable data on downstream migration of wild fish were available for 1980 and 1981 from counting fences on BQ (G. Ladouceur, Big Qualicum Fish Hatchery, Qualicum Beach, B.C., pers. comm.), and for a previous year (1979) on LQ (Lister, D. B. & Assoc. unpubl. rep. for the Department of Fisheries and Oceans, Vancouver, B.C.). Rough estimates of wild production were also calculated from annual spawner counts (Marshall et al. 1977) averaged over the past 30 yr using conversion factors (eggs per spawner, survival of eggs to fry or smolt) from Lister, D. B. & Assoc. (unpubl. rep. for Department of Fisheries and Oceans, Vancouver) and Minaker et al. (1979). I consider that estimates from spawner counts provide a satisfactory index, given that densities of juvenile salmon in streams with hatcheries are 2–3 orders of magnitude greater than those in unenhanced streams.

Census were undertaken on foot by two observers (with binoculars) working simultaneously in different zones of a single river system to cover the designated areas as quickly as possible. RC and BQ were censused regularly to 1 and 5 km upstream of their outlets, respectively, in 1980–82; LQ was censused to 1 or 2.5 km upstream in 1980 and to 5 km upstream in 1981; ENG was censused to 5 km upstream in 1981–82. Each census required 2–3 h. Censuses were conducted at intervals of $\leq$3 d in 1980 at various combinations of tide and time of day. During 1981, counts were made every 4 d, April–July, and less frequently in other months; census times were scheduled to provide maximum contrast between time of day and tide effects, since the semidiurnal tides follow a 2-wk cycle. Censuses were conducted each week, May–September 1982, and twice on BQ in March 1983.

Mergansers were counted without difficulty; they are conspicuous by colour and habit, vocalize when alarmed, thereby alerting the observer, and yet, are not especially wary. The greatest source of error in counts arose from birds on the wing which may not have been counted already. Fortunately, only a small proportion of birds (<5%) were recorded as flying during a typical census. To ensure abundance was not overestimated, the following convention was adopted for uncertain cases: birds flying into previously censused territory, from without, were recorded whereas those flying out of previously censused areas were not counted, and subtracted if others of matching sex and maturity were seen later in that direction of travel. Records were kept of sighting location to within 0.2 km, number and size of flocks, sex ratio, pairing and maturity status (where possible), activity, and the presence/absence of bands. Activity categories included foraging (sumerging head in search of prey or consuming prey), preening, interacting with conspecifics (display, chase, or copulation), sleeping, resting (alert but inactive), and flying. Foraging (or flying) activity was defined as the proportion of birds that were foraging (or flying) during the census. A flock was defined as a group of birds with a typical nearest-neighbour spacing <10 m. Using this scale as a guideline, flocks were usually obvious; a single, large flock was recorded wherever birds were very active so that spacing changed rapidly (e.g. during feeding frenzies).

Twenty-one mergansers were banded and marked individually with picric acid stain (1980) or colour-bands (1981). The birds were captured in size 15 mist nets (10-cm mesh) suspended across the stream. All but one were captured and released on BQ. Crop and gullet contents were obtained from 17 of 20 birds to which a 1% aqueous solution of antimony potassium tartrate had been administered at dosages of 10 cm^3/kg as described by Prŷs-Jones et al. (1974). Birds marked with stain in 1980 did not retain their marks past the wing moult in late summer and could not be recognized individually in the field in subsequent years.

Index of Juvenile Salmonid Density (JSI)

To analyze the aggregative response, I ranked releases of juvenile salmonids in order of profitability. This was straightforward where all fish released were of similar size. However, where several different salmonid species and size groups were released (e.g. BQ), some assumption about relative profitability was required. A simple pooled estimate of density would

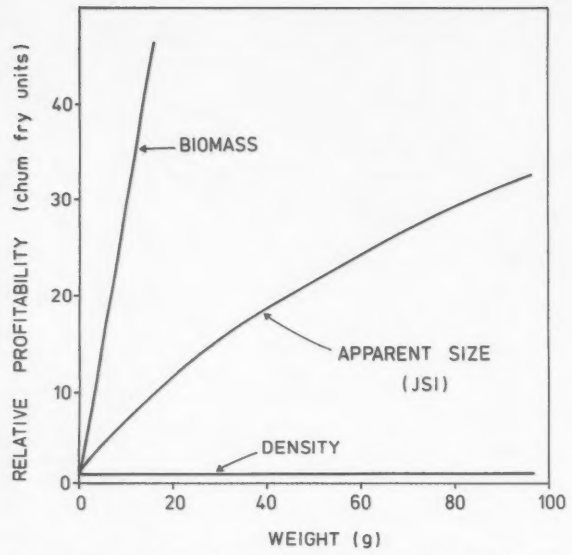

FIG. 2. Graphical comparison of the relative profitability (to mergansers) of different-sized salmonids under alternative size-selection assumptions. A chum salmon fry is typically 3.8 cm in length and 0.4 g in weight. A length–weight conversion derived from data reported by Bilton (1978) was used to compute the JSI in this example: weight (g) = 0.013 (length in cm)$^{2.898}$.

underestimate the contribution of larger fish (up to 90 g) in comparison with emergent fry (0.3–0.4 g); on the other hand, a pooled estimate of biomass would probably overestimate the availability of the larger fish. Capture rates of mergansers feeding on 2-g coho salmon (*Oncorhynchus kisutch*) fry and 40-g coho smolt have been evaluated (Wood and Hand 1985). Coho smolt were taken more frequently than fry but the selection could be explained on the basis of apparent size (i.e. cross-sectional area in the perceptual field) such that

$$(2)\quad \frac{a_f}{a_s} = \left(\frac{l_f}{l_s}\right)^2$$

where a_f and a_s are the rates of effective search for coho fry and smolt and l_f and l_s are the lengths of fry and smolt, respectively (see Wood 1984 for derivation). Accordingly, a juvenile salmonid index (JSI) was defined as

$$(3)\quad \text{JSI} = \sum_i n_i \left(\frac{l_i}{l_{cf}}\right)^2$$

where n_i is the number and l_i the length of salmonid type i and l_{cf}, the average length of an emergent chum salmon (*O. keta*) fry (3.8 cm). Unfortunately, model (2) could not be tested rigorously; the data available indicate $a_f/a_s \geq (l_f/l_s)^2$ (Wood 1984), which suggests that the JSI may exaggerate the importance of large fish. The relationship between selection by density, biomass, and JSI is illustrated in Fig. 2. Species differences were ignored.

Flock-Size Distributions

Additional data pertaining to flock formation and flock-size distributions were obtained during two consecutive experiments, one on RC during May 4–22, 1981, and another at the mouth of LQ on June 10–26, 1981. During the first experiment, stream enclosures were stocked with known densities of coho smolt (Wood and Hand 1985). The number of mergansers at the enclosure site was observed from a blind and recorded at 20-min intervals following a 3-h equilibration period. As foraging bouts were typically <10 min, each census was treated as an independent replicate of the flock-formation process.

In the second series of observations, flock sizes at the mouth of the LQ river were recorded at 20-min intervals from 10:30 to 13:00 and from 14:00 to 16:30 on June 10–12, 17, 19, and 24–26. Fish density was assumed to have been constant over this period. Overall merganser abundance on the estuary was recorded simultaneously from a raised blind.

A breeding pair was treated as a single "individual" in the following discussions of aggregation and flock size because members of the pair usually travel together; inclusion of both members would exaggerate the response and violate the assumption of independence in the EFS model. The typical flock size is calculated as $\Sigma x_i^2/\Sigma x_i$ where x_i is the size of the ith flock observed. It represents the flock size in which the "average" individual occurs (Jarman 1974). The parameters, k, of negative binomial distributions was estimated by the usual maximum likelihood method (e.g. Southwood 1978). The method of Brass (1958) was used for truncated negative binomial distributions as follows:

$$(4)\quad k = [p\bar{x} - f^*(1)]/(1 - p)$$

where $p = \bar{x}[1 - f^*(1)]/s^2$, $f^*(1)$ is the relative frequency of solitaries, and s^2 is the sample variance of flock size.

Results

Trends in Dispersion Unrelated to Salmonid Density

Mergansers were most abundant in the study area throughout March and April just after the peak period of northerly migration. The breeding pairs form early in the spring; egg-laying begins between early April and early June (Bellrose 1978) and continues for about 2 wk for an average-sized clutch (Erskine 1972). The pairs break up as soon as incubation begins and the males leave the vicinity to moult on protected estuaries or lakes (Bellrose 1978). Moulting males were rarely observed at any of the study sites. Most (22) were sighted on the extensive and sheltered tidal flats at the mouth of the Tsable River located 8 km northwest of RC. Downy young emerged between late May and August and tended to remain on or near their natal stream until able to fly at about 10 wk (Wood 1984).

Trends in abundance were generally similar from year to year within streams although they differed among streams (Fig. 3). Most day to day variation in estimates of abundance was probably due to movement of mergansers to and from the census area; I believe that only a minor proportion of this variation can be attributed to census error. The opposite trends in abundance among the three major rivers in Fig. 3 were associated with differences in salmonid production (see next section). Accordingly, I pooled the data for these rivers to reveal overall trends in the study area corresponding to seasonal breeding, moulting, and migratory activities (Fig. 4); the combined census data included most mergansers within a 20-km radius of the LQ estuary. Note that females with broods were excluded and a breeding pair was regarded as one bird to conform to assumptions of the EFS model.

The pooled estimate of abundance declined steadily from April through June. This seasonal decline began well before the pairs break up (June) and presumably reflects both mortality and

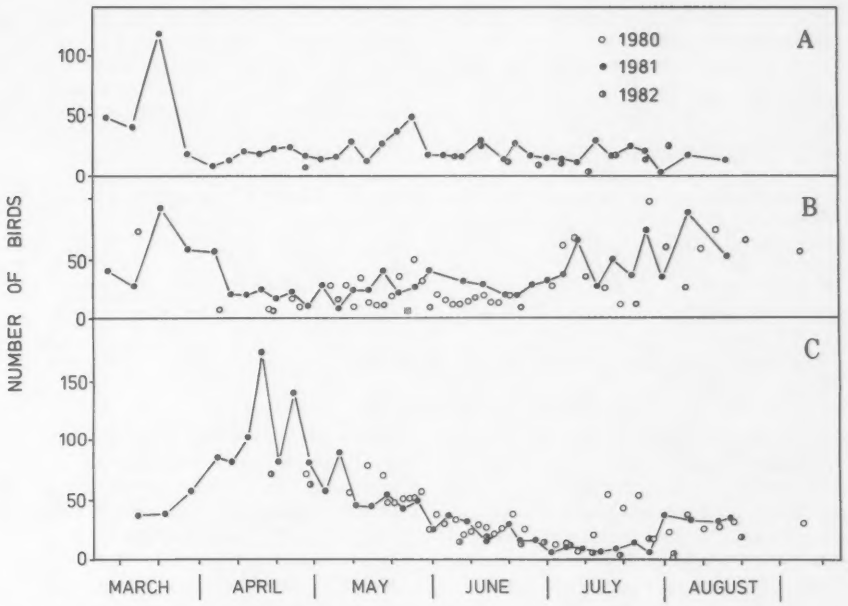

FIG. 3. Trends in abundance of mergansers on three adjacent river systems (tidal and freshwater zones). (A) Englishman River; (B) Little Qualicum River; (C) Big Qualicum River. Freshwater census of Little Qualicum in 1980 to 1 km upstream only; all other counts to 5 km upstream.

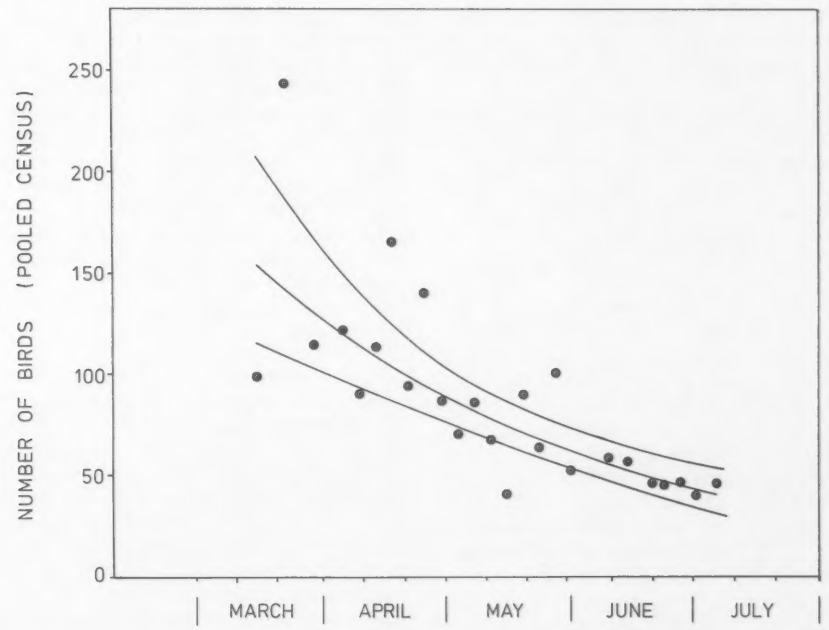

FIG. 4. Seasonal trend in abundance of mergansers in the study area based on pooled census data from three adjacent streams. The census covered tidal and freshwater (to 5 km upstream) zones of Englishman, Little Qualicum, and Big Qualicum rivers during 1981. Males of breeding pairs and females with broods were excluded. Regression is linear for log-transformed data with 95% C.I. for predicted values:

$$\log y = 5.04 - 0.012x, \quad n = 25, \quad r = -0.83$$

$$F_{1,23} = 50.0, \quad p < 0.001.$$

TABLE 2. Movements of mergansers banded on the Big Qualicum River in 1980–81 (figures in parentheses refer to mature male birds).

Date banded	Number banded	Minimum number of birds resighted (total)[a]			Minimum number of birds resighted outside BQ area		
		<30 d after release	>30 d after release	Overall	<30 d after release	>30 d after release	Overall
May 22 – June 13, 1980	7 (2)	4 (1)	2 (0)	5 (1)	4 (1)	2 (0)	5 (1)
August 18, 1980	2 —[b]	0 —	0 —	0 —	0 —	0 —	0 —
April 8–23, 1981	11 (4)	5 (1)	4 (0)	7 (1)	1 (0)	2 (0)	3 (0)
Total	20 (6)	9 (2)	6 (0)	12 (2)	5 (1)	4 (0)	8 (1)

[a]Some birds could not be distinguished after the first moult.
[b]Juveniles <10 wk.

northerly migration. The subsequent increase in abundance was due, in part, to the inclusion of juvenile birds, >50 d of age, that resemble adult female and immature birds. However, females and immatures (distinguished by their ability to fly) also became more numerous during July and August. Flocks of mixed adult and juvenile mergansers were characteristically large and cohesive during this period. Census counts were not correlated with either time of day, tide height, or cloud cover ($p > 0.25$, ANOVA).

Flight activity was much reduced in June compared with previous months ($p < 0.05$, χ^2). Flights were even less frequent during July and August, but this may be because juveniles and moulting birds could not always be distinguished in the censuses. No seasonal trends were evident with respect to foraging activity. Both flight activity and foraging activity varied widely among censuses. No significant influence could be attributed to time of day, tide height, or cloud cover.

Of 18 adults banded on BQ, at least 12 were resighted on a total of 53 occasions (Table 2). Almost half (45%) of resightings occurred more than 1 mo after release. Two individuals were resighted after 697 and 741 d at large. In both 1981 and 1982, one brood on BQ was accompanied by a female banded on the same stream in 1980; it is not known whether the banded female was the same individual in both years. All marked birds resighted in 1980 and 43% of those resighted in 1981 were observed to visit neighbouring rivers between LQ and RC. Several birds banded in May–June of 1980 were observed to make trips back and forth between adjacent streams. Despite more extensive census coverage and more frequent resightings, no birds banded in April 1981 were observed to make return trips to and from BQ. Moreover, these birds appeared to linger on the BQ system for a longer period after release before venturing to neighbouring streams. I attribute this to the availability of chum salmon fry which peaked during April 1981; chum fry were nearly 7 times more abundant in BQ than in any other Vancouver Island stream owing to the BQ spawning channel facility (Table 1).

Dispersion in Relation to Juvenile Salmonid Density

Trends in abundance

Differing trends in abundance among neighbouring streams evident in Fig. 3 can be explained to a large extent by differences in juvenile salmonid densities. Inverse relationships between BQ and LQ became obvious when the proportion of the pooled census data observed on each contributing stream was plotted against the total juvenile salmonid migration (in JSI units) for BQ in each month (Fig. 5). (Fish densities were summarized by month because precise information is not available regarding stream residence times for smolt released from the hatcheries.) Although BQ is the smallest of the three streams in terms of drainage area, flow, and length accessible to salmon, its annual production of salmonids was 2–5 times greater than that of LQ, the next most productive system. Females with broods were excluded, since their activities were confined to areas accessible to the flightless broods, and their inclusion would tend to obscure any movements between river systems.

The proportion of birds observed on freshwater as opposed to tidal waters was also correlated with the salmonid index within each of the three larger rivers studied (Fig. 6). Juvenile salmonids were rarely consumed by mergansers on salt water but comprised a large proportion of their diet on freshwater reaches of salmonid-producing streams and lakes (White 1957; Wood 1984). It is highly probable, therefore, that the relationships in Fig. 6 indicate an aggregative response to juvenile salmonids within river systems. An alternative interpretation might be that the correlation exists merely because nesting occurs on freshwater and happens to coincide with the downstream migration of salmonids. This is untenable, however, because during the periods of peak downstream migration (April–May), the majority of the merganser population present did not breed.

Flock-size predictions

The EFS model predicts that, on average, flocks will be larger on a more profitable patch than on a less profitable one provided sufficient time has elapsed for equilibrium to be achieved. If arrivals and departures occur at random, the EFS will also be a random variable. Caraco (1980) has shown that where the probability of arrival is a linear function of flock size and the probability of departure is constant, the EFS follows a negative binomial distribution. In terms of equation (1), if the expected value of the arrival function $E[a(F)] = \alpha + \beta F$ and the expected value for the departure function $E[d(F, P)] = \gamma$ at constant profitability (P), where α, β, and γ are probabilities for a Poisson process, then the EFS has a negative binomial distribution with parameters $\mu = \alpha/(\gamma - \beta)$ and $k = \alpha/\beta$.

Flock-size distributions observed at the enclosure site on RC are presented in Fig. 7 with respect to the maximum density of smolts available each day. As expected, flock size was, on average, significantly larger when 100–200 smolt were stocked

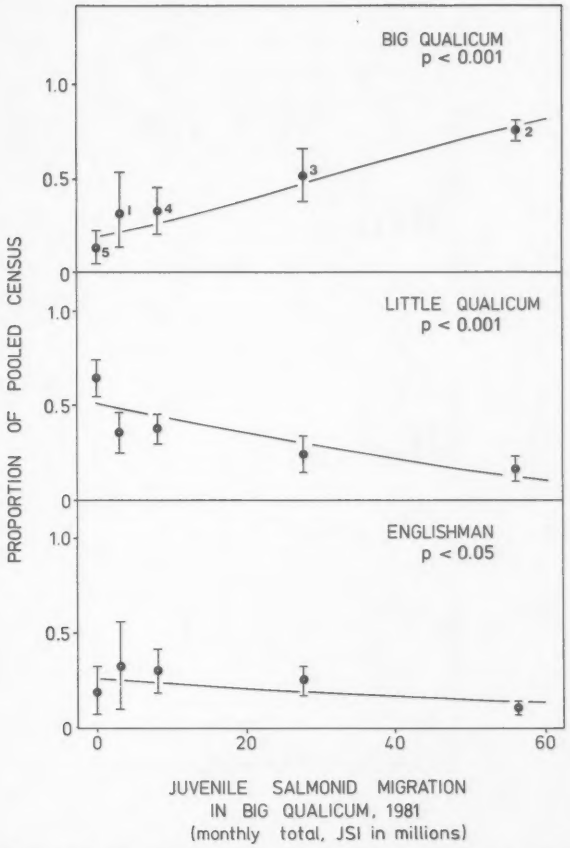

FIG. 5. Inverse relationships in merganser abundance on tidal and freshwater zones of three neighbouring rivers with respect to juvenile salmonid migration from Big Qualicum River. Numbers beside points refer to month of census: 1, March; 2, April; 3, May; 4, June; 5, July; 95% C.I. are given for normal distribution of arcsine-transformed data. Regressions are linear for transformed data:

BQ $y = 2.7 + 0.627x$, $n = 31$, $r = 0.81$
$F_{1,29} = 57.3$, $p < 0.001$

LQ $y = 45.9 - 0.463x$, $n = 31$, $r = -0.74$
$F_{1,29} = 35.2$, $p < 0.001$

ENG $y = 31.0 - 0.175x$, $n = 31$, $r = -0.38$
$F_{1,29} = 4.8$, $p < 0.05$.

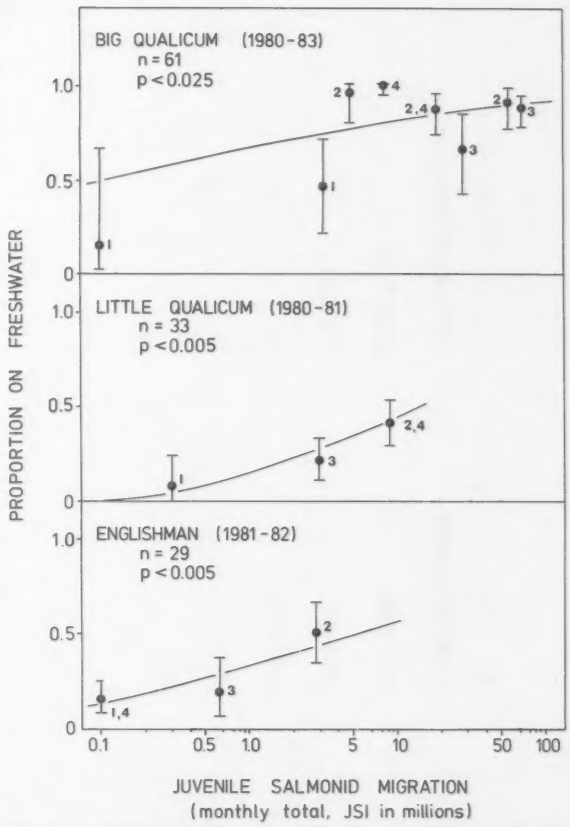

FIG. 6. Relationships between the proportion of mergansers on freshwater and juvenile salmonid migration by month, March–June. Freshwater census coverage was limited to 2.5 km above river mouth in order to include 1980 data from Little Qualicum River. *N* refers to the total number of census days; numbers beside points refer to month of census: 1, March; 2, April; 3, May; 4, June. Note log scale on abscissa; 95% C.I. are given for normal distribution of arcsine-transformed data. Regressions are linear for transformed data:

BQ $y = 54.09 + 9.36$ ($\log_{10}$ JSI in millions), $n = 61$, $r = 0.30$
$F_{1,59} = 5.92$, $p < 0.025$

LQ $y = 23.08 + 18.30$ ($\log_{10}$ JSI in millions), $n = 33$, $r = 0.49$
$F_{1,31} = 9.94$, $p < 0.005$

ENG $y = 35.46 + 13.16$ ($\log_{10}$ JSI in millions), $n = 29$, $r = 0.55$
$F_{1,27} = 11.48$, $p < 0.005$.

than when a maximum of 10–50 were available ($p < 0.001$, χ^2). Also, the flock-size distributions were not significantly different from those under the corresponding negative binomial distributions ($p > 0.25$, χ^2, for both low and high smolt density). However, the probability of arrival was probably not a strictly linear function of flock size at RC. First, the merganser population at RC was not very large in comparison with the flock being observed; maximum flock sizes of 6 and 15 mergansers were observed on the enclosures, and within 1 km of the enclosure site, respectively, during the experiments. Unless mergansers from farther afield were likely to visit the site, the probability of arrival would have been reduced as flock size increased. Second, arrival rates in the absence of a flock increased gradually over the course of the experiments as a result of experience (Wood 1985). To some extent this would have compensated for the seasonal decline in abundance expected over the period May 4–24. The significance of these deviations from linearity is not clear, but they could be expected to spread out the EFS distribution. Smolt density treatments were alternated randomly throughout the experimental period so that any trends in arrival rate did not invalidate that comparison.

Flock-size distributions at the mouth of LQ during mid- to late June exhibited a similar pattern and are summarized by week in Fig. 8. The availability of prey (mostly *Cottidae*) at the site was unknown but was assumed to be comparable over the 16-d period. Tidal action may have influenced prey availability but observation periods were scheduled 6 h apart so that both

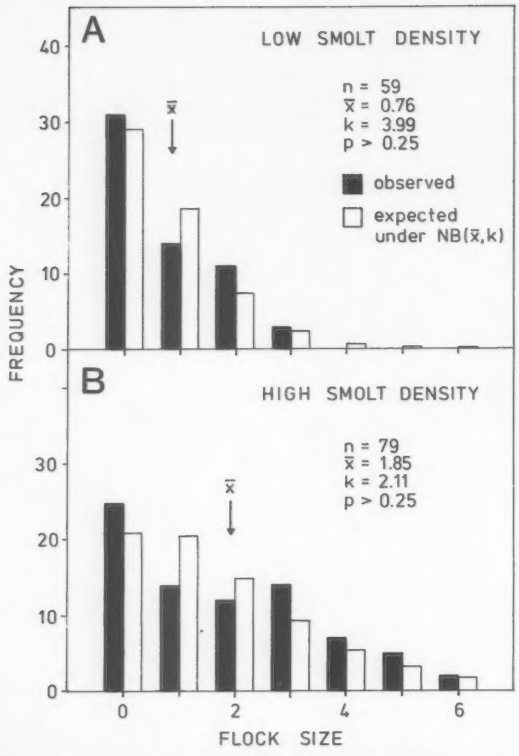

FIG. 7. Comparison of observed and expected distributions of equilibrium flock sizes with respect to coho smolt density stocked in enclosures at Rosewall Creek, 1981. Expected distribution is the negative binomial with parameters $\bar{x}$ and k. (A) Low smolt density (max. 10–50, $\bar{x}$ flock = 0.83, k = 16.1, $p > 0.05$); (B) high smolt density (max. 100–200, $\bar{x}$ flock = 1.92, k = 2.50, $p > 0.10$). Difference between densities is significant ($p < 0.001$, χ^2).

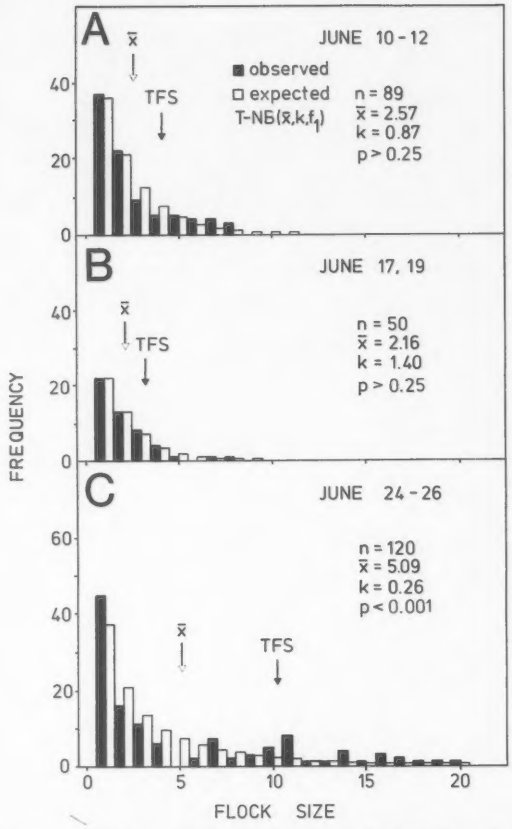

FIG. 8. Comparison of observed and expected distributions of equilibrium flock sizes at the mouth of the Little Qualicum River, 1981. Expected distribution is the truncated negative binomial with parameters $\bar{x}$ and k. (A) June 10–12 (n = 89, $\bar{x}$ flock = 2.57, k = 0.87, $p >$ 0.25); (B) June 17, 19 (n = 50, $\bar{x}$ flock = 2.16, k = 1.40, $p > 0.25$); (C) June 24–26 (n = 120, $\bar{x}$ flock = 5.09, k = 0.26, $p < 0.001$). Open arrows indicate means; solid arrows indicate typical flock size (TFS).

phases of the tide would be monitored. Moreover, tide action was virtually identical during the first (June 10–12) and third (June 24–26) periods, but reversed during the second (June 17, 19). Wind and cloud cover did not differ appreciably in any of the three periods. In contrast with the RC experiment, merganser density was high at LQ and arrival probabilities were close to linear, as illustrated by an experiment with decoys conducted simultaneously in an adjacent area upstream of the river mouth (Wood 1985). The flock-size distributions for the first two periods did not differ significantly from negative binomial distributions expected for the EFS ($p > 0.25$, χ^2). Flock sizes were substantially larger during the last week in June; moreover, their distribution did not correspond to a negative binomial distribution ($p < 0.001$, χ^2). Although the distribution of small flocks (≤6 birds) in the last week was similar to that in early weeks, larger flocks of up to 20 birds also assembled, creating a bimodal distribution. I infer that birds in the large flocks during late June were behaving gregariously — not independently as assumed by the EFS model. Most likely, this was a seasonal change associated with reduced flight activity noted during June–August.

I also compared flock-size distributions on fresh and tidal waters of BQ, LQ, and ENG in 1981, first, during the peak period of juvenile salmonid migration (April 1 – May 31), and second, for months subsequent to downstream migration (June 14 – September 14). Only gross comparisons were possible because so little was known about variations in profitability among different sites within the river systems. Both abundance and typical flock size were much greater on BQ during the salmonid migration than on either of the other larger streams. Only on BQ during the months of salmonid migration did typical flock sizes on freshwater exceed those on tidal waters. After the salmonid migration, the largest flocks and the greatest density of mergansers occurred on the LQ estuary. Typical flock sizes on tidal waters from mid-June to mid-September were larger than during April–May even though densities there were actually lower on ENG and BQ during the later months. Flocks became especially large (up to 56 mergansers) on the LQ during June 14 – September 14, which confirms the trend observed in Fig. 8.

Generalized EFS model for predicting aggregations

Although predictions of the EFS model were qualitatively correct for merganser flocks, the flock size distribution describes only part of the aggregative response. It is also necessary to know the *number* of flocks in a specified area. Because these

two quantities are interdependent, stochastic models are awkward to develop or apply (Cohen 1972; Morgan 1976). A simpler alternative is to regard the collection of flocks, itself, as a "superflock" or aggregation which grows and decays according to immigration and emigration by the remaining local population not included within the aggregation. In taking this approach, it was necessary to evaluate the following quantities: (a) the effective population size in the vicinity (N) — all birds equally likely to join an aggregation, (b) the probability that an individual (member of N) will approach the site of aggregation during an interval of 1 d (λ_1), (c) the probability that having approached the site, an individual will join the aggregation (λ_2), (d) the profitability at the site of aggregation (P), (e) the probability of departure for an individual member of the aggregation (d) given profitability P.

A deterministic, discrete-time model was constructed to make predictions about trends in the aggregative response of mergansers to hatchery releases of juvenile salmonids at RC and BQ. Expressions for assumptions (a) to (e) were as follows:

$$\text{(5)}\quad \text{Effective population size} = N_t = N_0 e^{-\omega t}$$

where t indicates time in days and $\omega = 0.012$. This expression was required because of the seasonal decline in abundance during the period of juvenile salmonid migration. The parameter ω was estimated by regressing the pooled census data for ENG, LQ, and BQ against time during March–June 1981; again each pair counted as one individual and females with broods were excluded ($p < 0.001$, F test, Fig. 4).

The probability of joining the aggregation, having already approached the site, was assumed to be of the same form as for joining a flock, since an approaching bird should be able to assess both the number and size of flocks from overhead.

$$\text{(6)}\quad \Pr(\text{joins}|\text{approach}) = \lambda_2 = \alpha + \beta A$$

where α and β are constants and A is the size of the aggregation. If the number of approaches per day follows a Poisson distribution with parameter λ_1, then the number of birds joining the aggregation will follow the compound Poisson distribution with parameter $\lambda_1\lambda_2$. The probability of joining the aggregation at least once in a day is therefore one minus the zero frequency as follows:

$$\text{(7)}\quad \Pr(\text{joining}) = a(A) = 1 - e^{-\lambda_1(\alpha+\beta A)}$$

where λ_1, α, $\beta > 0$ and $A > 0$. Parameters α and β were given values of 0.4 and 0.03, respectively, since the observed response to decoys ($\hat{\alpha} = 0.35$, $\hat{\beta} = 0.03$, Wood 1985) defined minimum values.

The number of departures was determined at the end of each day after all arrivals had taken place to facilitate computation. Because interference effects could not be demonstrated for mergansers foraging in flocks of up to 25 individuals (Wood and Hand 1985) and aggressive interactions appeared to play a small role (Wood 1985), the probability of departure was assumed to be independent of the size of the aggregation and to depend only on profitability. The average duration of visits was found to increase exponentially with fish density (Wood 1985), so the expected rate of departure (per bird) from an aggregation was assumed to follow the reciprocal relationship

$$\text{(8)}\quad E[d(P)] = \rho e^{-\nu P}$$

where ρ, $\nu > 0$ and $P > 0$. Profitability was assumed to be proportional to the JSI; the availability of nonsalmonid fishes was ignored. Residence times of smolt released from the hatcheries were known only approximately. At RC, steelhead (*Salmo gairdneri*) smolt were assumed to emigrate at a rate of 25% per day based on personal observations of how long they remained conspicuous in the stream. Estimates of smolt residence times for BQ were obtained from Mace (1983).

Parameters λ_1, ν, and ρ were assigned values which seemed plausible and provided an adequate scale of response in simulating responses to fish releases at RC in 1980. A satisfactory response was obtained with $\lambda_1 = 0.5$, $\rho = 0.8$, and $\nu = 0.05$. Since the BQ and RC systems differ greatly in size, it was also necessary to scale the effective population sizes and profitability estimates accordingly. The effective population size in the vicinity of BQ was taken to be the pooled census estimate for BQ, LQ, and ENG from Fig. 4 and $N_0 = 150$ where t_0 is March 20. For RC, N_0 was assumed to be the maximum number recorded in the vicinity following a large smolt release (31 birds on April 29, 1980) corrected for seasonal decline; hence, $N_0 = 31/e^{-40\omega} = 50$.

Profitability was scaled such that the predicted maximum aggregation size coincided closely with maxima for the observed trends in abundance for years with most census coverage (1980 at RC and 1981 at BQ). This required that the JSI be 25 times higher at BQ than at RC. Predictions for 1981 at RC and 1980 at BQ were generated without further adjustment of parameter values.

Predicted and observed trends are presented in Fig. 9 and 10 for RC and BQ, respectively. A close match of predicted and observed trends for one fish release at RC during 1980 was virtually assured by selecting appropriate λ_1, ν, and ρ values. It is encouraging, however, that predicted trends track observed trends so closely over *all* fish releases (Fig. 9A). Observed responses were less than predicted during June (days 72–102) in both 1980 and 1981. Merganser abundance was higher than expected during the enclosure experiments (days 42–80) in 1981 but the comparison is inappropriate because small numbers of smolt were made available at high density during this period (Fig. 9B).

Predicted trends at BQ in 1981 (Fig. 10B) matched the general features of the observed trends but again tended to overestimate abundance in late May through June. In this case, the predicted curve was fitted only by scaling profitability; other parameters are identical to those used in the RC predictions. The match in 1980 for BQ (Fig. 10A) is surprisingly good considering that the predicted trend was generated without any reference to the observed data, and that fish releases in 1980 differed considerably with respect to both size and time of release from those in 1981.

Discussion

The aggregation model, driven by a simple response to profitability, captured many qualitative features of the temporal dispersion of mergansers on salmon-producing streams. The predictive power of the model does not reflect on the truth or falsehood of the individual relationships comprising the model — these relationships have been validated independently by direct experiment or observation. Rather, errors in prediction should help to establish the relative importance of these relationships and to reveal whether other significant variables have been overlooked. More reliable predictions might have been possible had estimates of profitability been based on better information regarding prey size selection and the availability of nonsalmonid prey in estuaries. Nevertheless, the results are encourag-

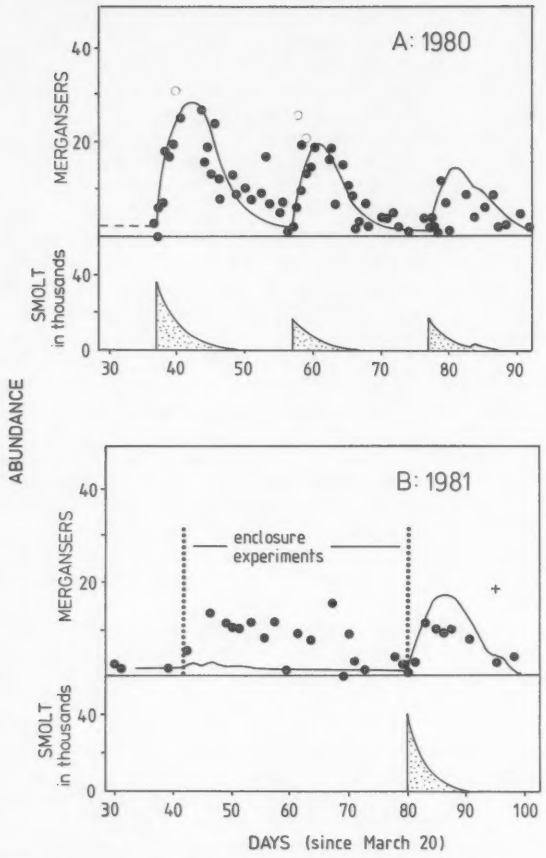

FIG. 9. Comparison of observed and predicted trends in abundance of mergansers at Rosewall Creek. (A) 1980; (B) 1981. Abundance is in units of the aggregation model (i.e. breeding males and females with broods excluded). ●, paired birds difficult to distinguish (three assumed); +, atypical, cohesive flock included (corresponding dot represents typical, small, scattered flocks only). Fish release: stippled, smolt; not stippled, fry.

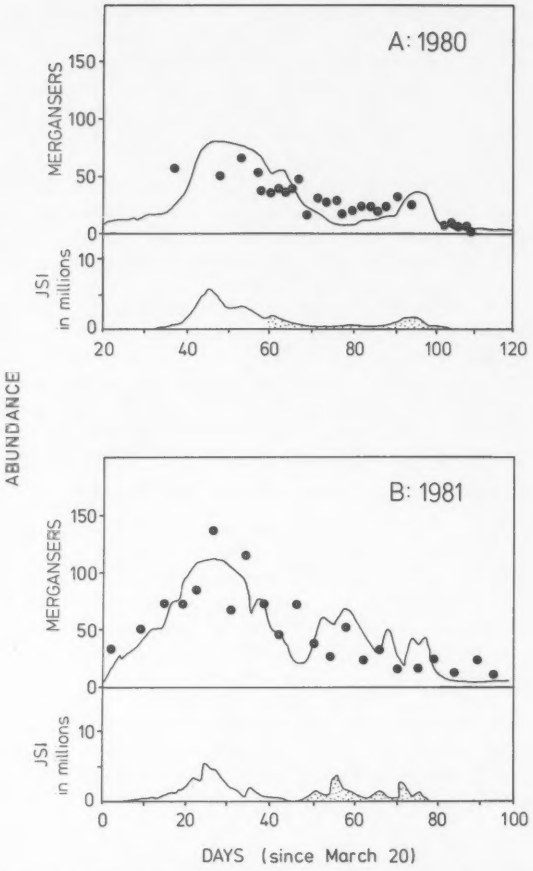

FIG. 10. Comparison of observed and predicted trends in abundance of mergansers at Big Qualicum. (A) 1980; (B) 1981. Abundance is in units of the aggregation model (i.e. breeding males and females with broods excluded). Fish release: stippled, smolts; not stippled, fry.

ing, for they suggest that the most important processes determining the aggregative response have been identified.

Throughout the preceding analysis, profitability was represented by a juvenile salmonid index (JSI) to provide a consistent assumption about size selection by mergansers. The precise formulation of the index is debatable, but obviously some index was required; moreover, the appropriate size-selection assumption must lie between the bounds of selection in proportion to biomass and selection in proportion to number (Fig. 2). Experimental evidence (Wood and Hand 1985) suggests that the JSI may overestimate the importance of large fish. If this is true, profitability was underestimated during the fry migrations (late March – early May) relative to the later smolt migrations. Such an error would tend to accentuate, not diminish, the relationships between profitability and merganser dispersion (Fig. 5 and 6). The JSI assumption had virtually no effect on the RC predictions because all fish released from the RC hatchery were of comparable size excepting a small release of large (1-g) chum fry late in 1980. However, predicted trends on BQ might be inflated late in the season relative to trends during the earlier migrations.

Arrival rate was assumed to depend on effective population size and a local enhancement term determined by flock (or aggregation) size. Population size was estimated empirically for both the RC and BQ areas. By adopting individual estimates for the two areas, rather than a single estimate for the entire coastline, I have assumed that other factors besides fish density influence dispersion. Indeed, mergansers were always more abundant on BQ than RC even during October and November, long after juvenile salmon migration had ceased, and subsequent to departure of most migrating mergansers $\bar{x} = 15.0 \pm 2.6$ (± 1 SE) on BQ and 2.5 ± 2.5 on RC). No attempt has been made to identify the significant factors but it seems likely that physical characteristics of the streams such as width, length, and current velocity would be relevant cues easily perceived by mergansers.

The decline in abundance from April through June, used in predicting aggregation size (i.e. equation (5)), was estimated from combined (1981) data for BQ, LQ, and ENG. Thus, one might argue that the predicted trends for BQ in 1981 were not

independent of the observations. But the exponential decline in abundance over the full season was apparent only in the composite data. Furthermore, the reciprocal relationships between BQ, LQ, and ENG indicate that other processes besides continuous decline operate within the BQ system. The same decline parameter, based on the composite 1981 data, was used to generate predictions for RC and for BQ in 1980; hence, these predictions were independent of the observed trends.

The local enhancement term in (6) controlled the initial rate of accumulation (i.e. speed of response) following an increase in profitability. But the probability of joining the aggregation, given an approach, reached certainty for aggregations exceeding 20 birds. Thus, the local enhancement term had a significant effect only for small aggregations such as those on RC and those following intervals of low profitability on BQ.

The probability of approach, λ_1, was the most critical parameter determining number of arrivals and, consequently, the responsiveness of the aggregation to increased profitability. It is likely that reduced flight activity, such as observed from June to September, would decrease the rate of approach. Because λ_1 was constant in the simulations, predicted arrival rates and, hence, aggregation size may have been excessive late in the season (after day 80 in simulations). Indeed, the discrepancy between predicted and observed trends could be reduced in simulations for RC and for BQ in 1981 by decreasing λ_1 in accordance with observed flight activity.

Two parameters determined the probability of departure: the first, ρ, merely scaled the departure rate whereas the second, ν, governed the response to profitability. Scaling profitability, P, independently for BQ and RC was equivalent to fitting ν individually. The residence time of fish following release determined how P (or equivalently ν) changed over time. Accordingly, one must ask how much of the fit in Fig. 9 and 10 was due to assumptions about changes in profitability and how much results from the model having the correct dynamics. Profitability is related to both prey density and the vulnerability of prey. Because BQ and RC differ greatly in size, especially in stream volume below the hatchery, it is not surprising that more fish were required to produce a given response in BQ than in RC — some scaling was necessary. The BQ hatchery is located approximately twice as far upstream as the RC hatchery, and BQ appears to be 2–3 times wider and perhaps 3 times as deep, on average, than RC. At a very rough estimate, a given quantity of fish would be up to 18 times more concentrated in RC than in BQ. Thus, the scaling factor of 25 adopted to fit the model is similar to that expected on the basis of dilution alone, although other factors such as cover and turbidity may influence vulnerability. Excellent cover is available in five or six pools below the hatchery on RC but there are lengthy intervening sections of shallow riffles where cover for large fish is poor. Moreover, the water in RC is particularly clear. Water depth in BQ appears to be more consistent throughout its length below the hatchery so that fish may pass through fewer bottlenecks where they become especially vulnerable to predation.

Freshwater residence time of salmonids following release varies among species (H. T. Bilton, Pacific Biological Station, Nanaimo, B.C., pers. comm.) and with size and date of release (Bilton et al. 1982). My observations of the decline in abundance of steelhead smolt in reference pools in RC and of the incidence of coded-wire tags in otter spraints indicate that while most fish left during the first few days, some smolt remained in the stream for up to a month following release. In the RC simulations, I have assumed that fish emigrate at the rate of 25% per day; in other words, 50% would have left the stream within the first 3 d but 0.1% would still remain after 26 d. Although this exponential model describes my observations adequately, it is equally possible that the majority of fish emigrated during the first few days but that those remaining were depleted by predators at a constant rate (i.e. linear decrease in abundance). Smolt densities following release in BQ have been calculated by Mace (1983) who found that emigration of coho and chinook salmon (*O. tshawytscha*) was best described in two phases — an exponential decline in abundance during the first two-thirds of the migration followed by a slower, linear decline during the remainder of the migration. If this was also true for steelhead trout at RC, profitability would have declined more rapidly, immediately following a release, but remained at a low to intermediate value for a longer period than has been assumed; under the EFS model, aggregations would have peaked sooner but dispersed less quickly, and consequently, predicted trends would have matched the observed trends in Fig. 9 more closely.

Predictions of the aggregation model were most influenced by variations in parameters λ_1 and ν. Isopleths for residual variance (mean sum of squared errors) are plotted for a range of values of λ_1 and ν in Fig. 11A. The plot indicates that a wide range of combinations of these two parameters fit the data equally well; in other words, the absolute value of either parameter is not critical to the model's performance.

A second technique for evaluating robustness of a model is to draw parameter values randomly from prior distributions determined by independent data or subjective estimates (D. Ludwug, University of British Columbia, Vancouver, B.C., pers. comm.). With respect to the present model, three parameters, λ_1, ν, and ρ, were of primary concern — the others were relatively well defined by independent observation. Uniform prior distributions were assigned to each within the following limits: one-half to twice the value of λ_1 and ν giving best fit, i.e. 0.25–1.0 and 0.035–0.14, respectively, and 0.6–1.0 for ρ (the probability of leaving the vicinity within 1 d given zero profitability). The results of five trials with combinations of λ_1, ν, and ρ drawn randomly from within these intervals are illustrated in Fig. 11B. All trajectories lie within the shaded region; four of the five runs were statistically significant ($p < 0.001$, F test). Hence, I conclude that the aggregation model is robust to variations in parameter values. Yet, there appears to be a systematic decline in the aggregative response throughout the season that was not captured by the model regardless of parameter values. I attribute this anomaly to a seasonal change in ranging distance or social behaviour as evidenced by decreased flight activity and larger flock sizes during the summer months.

Two problems are likely to be encountered in applying the model outside the context of this study. First, mergansers demonstrated an ability to learn the location of profitable feeding areas (Wood 1985); the effect of previous experience was ignored in the model because the learned response was slow in comparison with the time-scale for fluctuations in prey density. This condition is satisfied only during periods when fish are being released from hatcheries frequently, but not continuously. In general, the effect of experience cannot be ignored. Second, the performance of the model is dependent on mergansers dispersing independently in their search for favourable feeding areas. This condition appears to be satisfied during the breeding season but it is probably violated at other times of the year. Even so, the aggregation model is a potentially useful tool

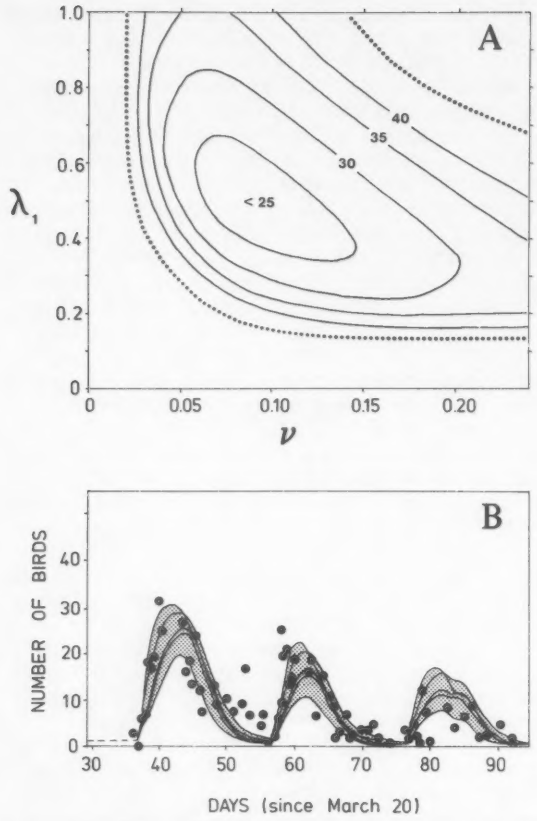

FIG. 11. Sensitivity of the aggregation model to variations in parameter values with respect to predictions at Rosewall Creek, 1980. (A) Contour plot of mean sum of squared residuals with respect to values of parameters λ_1 (arrival) and ν (departure); the fit to data is highly significant ($p < 0.001$) for mean sum of squared values < 36; residual sum of squares = total sum of squares along dotted line. (B) Predicted trends from combinations of parameters λ_1, ν, and ρ drawn randomly from uniform prior distributions.

for evaluating alternative hatchery-release schedules in order to minimize predation by mergansers.

Further research is required to extend flock size models to multiple patches. Recent studies have shown that mallards (*Anas platyrhynchos*) (Harper 1982) and feral pigeons (*Columba livia*) (Lefebvre 1983) distribute themselves in groups among patches such that each experiences similar feeding success (the "ideal free" distribution, Fretwell 1972). However, in these experiments, patches were close together and relative profitability could be assessed rather easily. On a larger spatial scale, such as between river systems, sampling is necessarily more expensive; since stakes are higher, the consequences of patch choice decisions should have a greater effect on fitness. The extension of theory, originally derived on small spatial scales, to larger scales is a necessary process in testing our understanding of behaviour and population ecology. Moreover, such investigation is necessary to achieve results of practical benefit for the management of natural resources.

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References

BARNARD, C. J. 1980. Equilibrium flock size and factors affecting arrival and departure in feeding house sparrows. Anim. Behav. 28: 503–511.

BELL, G. P. 1980. Habitat use and response to patches of prey by desert insectivorous bats. Can. J. Zool. 58: 1876–1883.

BELLROSE, F. C. 1978. Ducks, geese and swans of North America. 2nd ed. Stackpole Books, Harrisburg, PA.

BILTON, H. T. 1978. Returns of adult coho salmon in relation to mean size and time at release of juveniles. Fish. Mar. Serv. Tech. Rep. 832: 73 p.

BILTON, H. T., D. F. ALDERDICE, AND J. T. SCHNUTE. 1982. Influence of time and size at release of juvenile coho salmon (*Oncorhynchus kisutch*) on returns at maturity. Can. J. Fish. Aquat. Sci. 39: 426–447.

BRASS, W. 1958. Simplified methods of fitting the truncated negative binomial distribution. Biometrika 45: 59–68.

CARACO, T. 1980. Stochastic dynamics of avian foraging flocks. Am. Nat. 115: 262–275.

COHEN, J. E. 1972. Markov population processes as models of primate social and population dynamics. Theor. Popul. Biol. 3: 119–134.

ERSKINE, A. J. 1972. Populations, movements and seasonal distribution of mergansers. Can. Wildl. Serv. Rep. Ser. No. 17: 35 p.

FRETWELL, S. D. 1972. Populations in a seasonal environment. Monogr. Popul. Biol. 5: 218 p.

HARPER, D. G. C. 1982. Competitive foraging in mallards: 'ideal free' ducks. Anim. Behav. 30: 575–584.

JARMAN, P. J. 1974. The social organization of antelope in relation to their ecology. Behaviour. 58: 215–267.

KREBS, J. R. 1974. Colonial nesting and social feeding as strategies for exploiting food resources in the great blue heron (*Ardea herodias*). Behaviour 51: 99–131.

LEFEBVRE, L. 1983. Equilibrium distribution of feral pigeons at multiple food sources. Behav. Ecol. Sociobiol. 12: 11–17.

MACE, P. M. 1983. Bird predation on juvenile salmonids in the Big Qualicum Estuary, Vancouver Island. Can. Tech. Rep. Fish. Aquat. Sci. 1176: 79 p.

MARSHALL, D. E., R. F. BROWN, V. D. CHAHLEY, AND D. G. DEMONTIER. 1977. Preliminary catalogue of salmon streams and spawning escapements of Statistical Area 14 (Comox–Parksville). Fish. Mar. Serv. (Pac. Reg.) PAC/D-77-1: 176 p.

MINAKER, B. A., F. K. SANDERCOCK, AND L. I. BALMER. 1979. Big Qualicum River report, 1974–1975. Fish. Mar. Serv. MS Rep. 1528: 131 p.

MORGAN, B. J. T. 1976. Stochastic models of grouping changes. Adv. Appl. Prob. 8: 30–57.

O'CONNOR, R. J., AND R. A. BROWN. 1977. Prey depletion and foraging strategy in the oystercatcher (*Haematopus ostralegus*). Oecologia 27: 75–92.

PHELAN, F. J. S., AND R. J. ROBERTSON. 1978. Predatory responses of a raptor guild to changes in prey density. Can. J. Zool. 56: 2565–2572.

PRŸS-JONES, R. P., L. SCHIFFERLI, AND D. W. MACDONALD. 1974. The use of an emetic in obtaining food samples from passerines. Ibis 116: 90–94.

READSHAW, J. L. 1973. The numerical response of predators to prey density. J. Appl. Ecol. 39: 342–351.

RUSCH, D. H., E. C. MESLOW, P. D. DOERR, AND L. B. KEITH. 1972. Response of great-horned owl populations to changing prey densities. J. Wildl. Manage. 36: 282–292.

SALYER, J. C. II, AND K. F. LAGLER. 1940. The food and habits of the American

merganser during winter in Michigan, considered in relation to fish management. J. Wildl. Manage. 4: 186–219.

SMITH, J. N. M., AND H. P. A. SWEATMAN. 1974. Food searching behaviour of titmice in patchy environments. Ecology 55: 1216–1232.

SOUTHWOOD, T. R. E. 1978. Ecological methods. 2nd ed. Chapman and Hall, London. 524 p.

THORPE, W. H. 1963. Learning and instinct in animals. 2nd ed. Methuen, London.

VILLAGE, A. 1982. The home range and density of kestrels in relation to vole abundance. J. Anim. Ecol. 51: 413–428.

WHITE, H. C. 1957. Food and natural history of mergansers on salmon waters in the maritime provinces of Canada. Bull. Fish. Res. Board Can. 116: 63 p.

WOOD, C. C. 1984. Foraging behaviour of common mergansers (*Mergus merganser*) and their dispersion in relation to the availability of juvenile Pacific salmon. Ph.D. thesis, University of British Columbia, Vancouver, B.C. 307 p.

1985. Food-searching behaviour of the common merganser (*Mergus merganser*) II: Choice of foraging location. Can. J. Zool. 63. (In press)

WOOD, C. C., AND C. M. HAND. 1985. Food-searching behaviour of the common merganser (*Mergus merganser*) I: Functional responses to prey and predator density. Can. J. Zool. 63. (In press)

Influence of the Insecticide Diflubenzuron (Dimilin) on the Growth of Marine Diatoms and a Harpacticoid Copepod in Culture

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Antia, N. J., P. J. Harrison, D. S. Sullivan, and T. Bisalputra. 1985. Influence of the insecticide Diflubenzuron (Dimilin) on the growth of marine diatoms and a harpacticoid copepod in culture. Can. J. Fish. Aquat. Sci. 42: 1272–1277.

Diflubenzuron (Dimilin) was tested, in the concentration range 0.1–5000 $\mu g \cdot L^{-1}$, for possible injurious effects on the growth and photosynthesis of three chitin-producing (*Thalassiosira weissflogii*, *T. nordenskioldii*, *Cyclotella cryptica*) and one nonchitinaceous (*Skeletonema costatum*) diatoms. For comparison, the effects of the pesticide were also examined on adult survival and juvenile development of the harpacticoid copepod *Tigriopus californicus*. While the development of the copepod was hindered at concentrations of the order of 1–10 $\mu g \cdot L^{-1}$, the diatoms were barely affected by Dimilin even at the highest concentration tested (5 $mg \cdot L^{-1}$). We conclude that Dimilin acts specifically on insects and crustaceans as a larvicide by interfering with chitin deposition into cuticles during juvenile development through ecdysis. The lack of effect from Dimilin on the chitin-producing diatoms has suggested that the insecticide may not inhibit chitin biosynthesis per se as was previously believed, but that it presumably deregulates one or more of the larval postsynthetic processes responsible for chitin integration into cuticles.

On a étudié le potentiel d'incidences néfastes de concentrations de Dimilin variant de 0,1 à 5 000 $\mu g \cdot L^{-1}$ sur la croissance et la photosynthèse de trois diatomées secrétant de la chitine (*Thalassiosira weissflogii*, *T. nordenskioldii*, *Cyclotella cryptica*) ou non (*Skeletonema costatum*). À des fins de comparaison, on a aussi examiné l'incidence du pesticide sur la survie des adultes et le développement des juvéniles du copépode *Tigriopus californicus* de la famille Harpactidés. Quoique le développement de ce dernier ait été retardé à des concentrations variant de 1 à 10 $\mu g \cdot L^{-1}$, les diatomées étaient à peine touchées par le Dimilin même à la concentration la plus élevée (5 $mg \cdot L^{-1}$). Les auteurs formulent la conclusion que le Dimilin agit comme larvicide spécifique contre les insectes et les crustacés en perturbant le dépôt de chitine dans le cuticule au cours du développement des juvéniles par ecdysis. L'absence d'effet du Dimilin sur les diatomées productrices de chitine porte à croire que l'insecticide n'empêche pas la biosynthèse de la chitine per se comme on le croyait, mais qu'il dérègle vraisemblablement un ou plusieurs des processus post-synthèse chez la larve, responsables de l'assimilation de la chitine dans les cuticules.

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The insecticide Diflubenzuron, better known by its trade name Dimilin, is a urea derivative (with the chemical structure shown in Fig. 1), which was designed to control insect infestations by interfering with cuticle deposition during insect growth through moulting or ecdysis (Jakob 1973; Mulder and Gijswijt 1973). At the biochemical level, Dimilin is believed to inhibit chitin synthesis during endocuticle formation (Post et al. 1974; Deul et al. 1978). While desirable for insect control, such antagonistic biochemical action becomes a matter of ecological concern where the insecticide could injure the survival of nontarget flora and fauna dependent on chitin production for their development. This concern applies especially to the marine environment, where several trophic levels of the food chain are known to synthesize chitin (Muzzarelli 1977). Sprays of the insecticide are expected to be used in estuarine and coastal areas for mosquito or other insect pest control (Jakob 1973).

Apart from the well-known case of chitinaceous crustaceans such as copepods and crabs, some planktonic diatoms are known to produce chitin fibres which appear to be essential for their development (McLachlan et al. 1965; Falk et al. 1966; McLachlan and Craigie 1966; Blackwell et al. 1967; Dweltz et al. 1968; Conover 1975; Herth and Zugenmaier 1977; Herth and Barthlott 1979; Herth 1979). Concern about the environmental impact of Dimilin has led to several investigations of the effects of this insecticide on copepods (Tester and Costlow 1981), brine shrimp (Cunningham 1976), crabs (Christiansen et al. 1978; Costlow 1979; Christiansen and Costlow 1982), zooplankton and fish (Colwell and Schaefer 1980), and even blue-green algae (Wurtsbaugh and Apperson 1978). Surprisingly, our

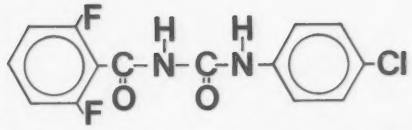

FIG. 1. Structural formula of Diflubenzuron (Dimilin) defining the insecticide in chemical terminology as 1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea or 1-(2,6-difluorobenzoyl)-3-(4-chlorophenyl)-urea.

survey of the literature has revealed no such studies on diatoms irrespective of their capacity for chitin production. Accordingly, we undertook a comparative investigation of the effects of Dimilin on the growth of a nonchitinaceous and three chitinaceous marine diatoms as well as a harpacticoid copepod serving as a crustacean control.

Materials and Methods

Diatoms

Cyclotella cryptica Reimann, Lewin and Guillard (clone T-13L) and *Thalassiosira (fluviatilis) weissflogii* (Grunow) Fryxell and Hasle (clone "Actin") were chosen as representatives of the diatoms known to produce chitin (McLachlan et al. 1965; Falk et al. 1966; McLachlan and Craigie 1966; Blackwell et al. 1967). Specimen cultures of both these diatoms were kindly supplied by Dr. J. C. Lewin. *Thalassiosira nordenskioldii* Cleve (an isolate from the Strait of Georgia, B.C., supplied by the Northeast Pacific Culture Collection (NEPCC) of the Oceanography Department, University of British Columbia, Vancouver, B.C., was chosen as a case of a diatom believed (but not yet proven) to produce chitin; this belief is based on scattered observations from various investigators that the production of chitinaceous fibrils, extracellular "spines," or threads linking cells in a colony may be common to all species of *Thalassiosira* (Hasle 1978, 1979; Herth 1979; J. S. Craigie, Atlantic Regional Laboratory, NRC Halifax, pers. comm.). *Skeletonema costatum* (also provided by NEPCC) was chosen to represent nonchitinaceous, yet chain-forming, diatoms. All the diatoms were autotrophically grown in unialgal batch culture at 18–20°C, under cool-white fluorescent light (irradiance approximately 80 $\mu E \cdot m^{-2} \cdot s^{-1}$), using a 14 h light : 10 h dark cycle. The culture medium consisted of filter-sterilized natural seawater (salinity approximately 28‰) enriched with "ES" nutrients according to Harrison et al. (1980).

Dimilin was dissolved in acetone on account of its poor solubility ($<0.2\ mg \cdot L^{-1}$) in water. The acetone tolerance of each diatom was previously determined by adding up to 0.5 mL acetone to 100 mL of culture medium and measuring algal growth by in vivo fluorescence (Strickland and Parsons 1972). After adding the appropriate Dimilin concentration to the culture medium, each culture flask was inoculated with the diatom under test and incubated for 11–14 d under the growth conditions described above. Growth was monitored from periodic cell counts and in vivo fluorescence. Appropriate controls (excluding Dimilin) were taken with and without the amount of acetone used to solubilize the pesticide. In addition, photosynthesis was measured by adding $NaH^{14}CO_3$ (9.25–18.5 kBq) to a grown culture (40 mL) previously treated with Dimilin and measuring ^{14}C-assimilation after 2–4 h of incubation in light (Strickland and Parsons 1972).

Copepod

The intertidal harpacticoid *Tigriopus californicus* was maintained in culture (on a fish-protein diet) from a stock originally supplied by Dr. A. G. Lewis (Oceanography Department, University of British Columbia). Using adult copepods, acetone tolerance was tested in the range 0.02–1.54% acetone added to seawater and found to be satisfactory at levels of 0.15–0.20% (v/v) acetone for the Dimilin tests. In a typical test series, various Dimilin concentrations were added to seawater in vials holding seven or eight adult copepods and periodic observations were made on the condition of the pesticide-exposed animals for several weeks. The characters monitored included adult viability, the number of females developing egg-sacs, and the presence or absence of nauplii.

Results

The ^{14}C-photosynthesis measurements at 18°C showed little effect on all the diatoms from Dimilin additions up to $1\ mg \cdot L^{-1}$ (Table 1). At a pesticide concentration of $5\ mg \cdot L^{-1}$, *Cyclotella* was still barely affected, but the other diatoms indicated significant reduction in photosynthesis up to approximately 70–80% of the controls taken without any additions. Interestingly, the acetone additions alone produced considerable stimulation of photosynthesis in *Skeletonema*, but they were without effect on *T. nordenskioldii* or slightly depressed photosynthesis in the cases of *T. weissflogii* and *C. cryptica* (Table 1). Surprisingly, it was found that lower temperature mitigated Dimilin toxicity, since the photosynthesis measurements made at 5°C were virtually similar at all the Dimilin concentrations tested (Table 1).

When the Dimilin was tested for effects on growth of *S. costatum*, it appeared to strongly stimulate both the growth rate and in vivo fluorescence yield at all concentrations tested relative to the control with no additions (Fig. 2). Similar growth tests on *T. nordenskioldii*, however, showed little stimulation and no retardation of growth rate or fluorescence yield irrespective of Dimilin concentration up to a maximum of $5\ mg \cdot L^{-1}$ (Fig. 2). It thus appeared that, despite a small degree of inhibition of photosynthesis, Dimilin caused little injury to the diatoms tested, irrespective of their capacity for chitin production. We have not yet examined the pesticide's effects on the diatom ultrastructure, but the light microscope observations indicated no gross aberrations in the diatom morphology.

In contrast with the diatoms, the copepod *T. californicus* showed injurious effects from exposure to Dimilin even at a concentration as low as $1\ \mu g \cdot L^{-1}$ (Fig. 3). Whereas the control containing no Dimilin showed sizable increase in the number of adults from continuing nauplii production over the period studied, the Dimilin-exposed adult copepods steadily diminished with no evidence of nauplii production. From another experiment (Table 2), it was confirmed that the control vials contained numerous nauplii and the adult population was increasing by the 31st day. The acetone control vials showed a slight delay in reproductive output and one of the replicate vials failed to produce nauplii. The copepods in the vials containing Dimilin at $0.1\ \mu g \cdot L^{-1}$ all produced nauplii and reproduced at a similar rate to the control (Table 2). For Dimilin at the $1\ \mu g \cdot L^{-1}$ level, nauplii were eventually observed in all vials (by day 45); however, only one of the replicates succeeded in producing adults from the nauplii. At the $5\ \mu g \cdot L^{-1}$ level, nauplii were observed only on days 25 and 31 of exposure; however, they appear to have perished, since they were not observed again.

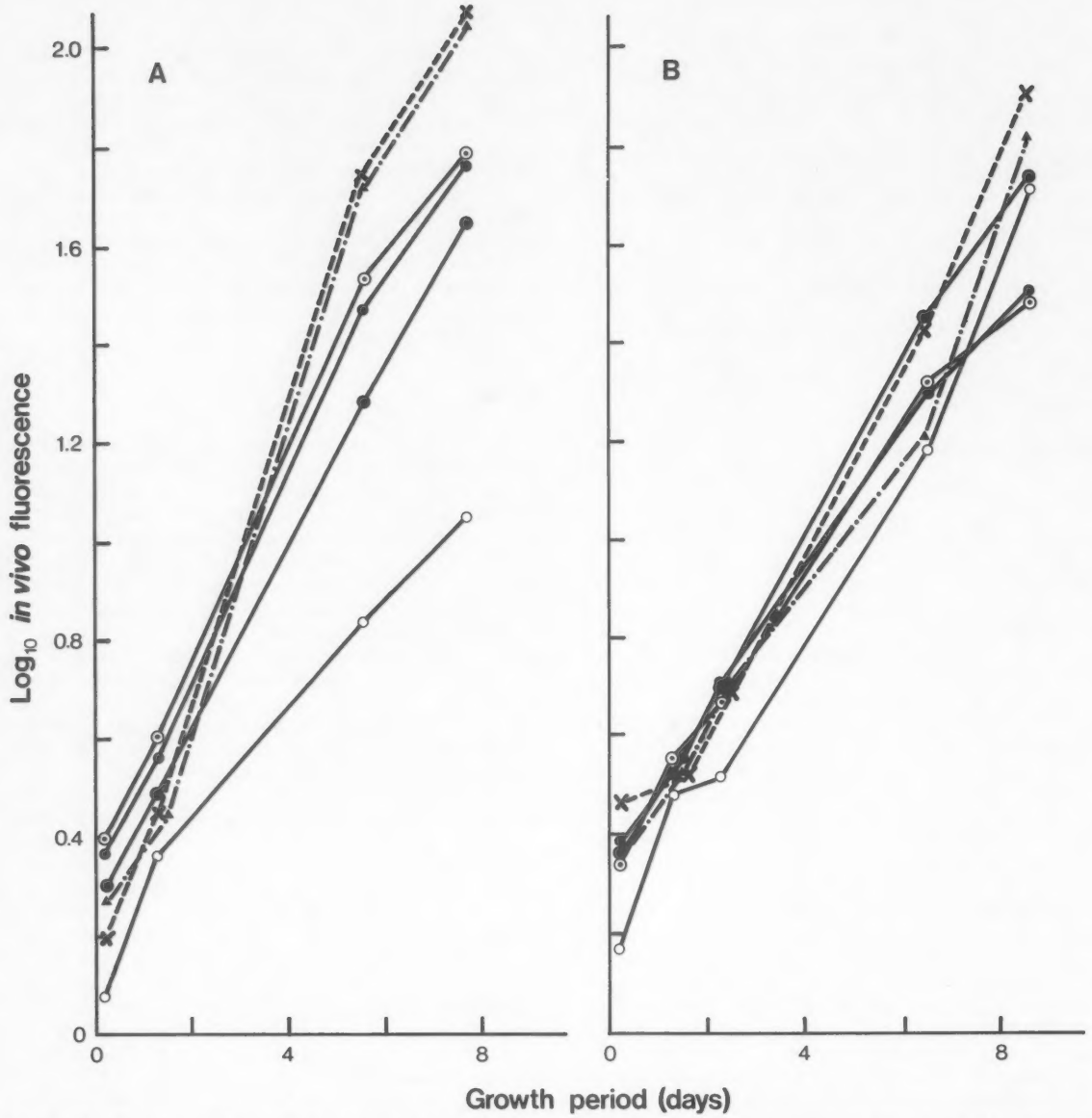

FIG. 2. Growth curves of diatoms (A) *S. costatum*, and (B) *T. nordenskioldii* in the presence of Dimilin concentrations ($\mu g \cdot L^{-1}$) as shown. ○, control (no Dimilin); ⊙, acetone control without Dimilin; ●, plus 10 Dimilin; ●, 100 Dimilin; ▲, 1000 Dimilin; ×, 5000 Dimilin.

Regarding the Dimilin treatments at 10 and 100 $\mu g \cdot L^{-1}$, nauplii were never observed and the copepod populations were moribund (Table 2).

In view of a previous report on the persistence of Dimilin in brackish water (Christiansen and Costlow 1980), we investigated the effectiveness of a Dimilin stock solution aged 18 d in acetone and an additional 10 d in seawater before introduction of the copepods (Table 3). The number of juveniles appearing in the Dimilin-treated samples was used as an index of Dimilin instability, since the previous experiments had shown that even at 1 $\mu g \cdot L^{-1}$, the pesticide tended to suppress nauplii formation. All the Dimilin-treated samples supposedly containing 1–10 $\mu g \cdot L^{-1}$ showed good adult survival and nauplii production in about 16–23 d of treatment, with little difference from the controls lacking Dimilin (Table 3). This observation suggested considerable decomposition of the Dimilin from approximately 4–5 wk of placement in seawater, which rate of decomposition appeared to be almost twice as rapid as that previously reported for estuarine seawater (Christiansen and Costlow 1980).

Discussion

Under the test conditions used in this study, Dimilin was clearly injurious to juvenile (nauplii) production by the harpac-

TABLE 1. Diatom photosynthesis ($NaH^{14}CO_3$ uptake) measured in the presence of Dimilin added as an acetone solution.

Diatom species	Temperature of measurement (°C)	Photosynthesis (mg $C \cdot m^{-3} \cdot h^{-1}$) values[a] for the Dimilin concentrations (mg $\cdot L^{-1}$) shown:				
		Control[b]	0[c]	0.1	1.0	5.0
S. costatum	18	1571	2004	1692	1515	1272
	5	393	517	532	579	512
T. nordenskioldii	18	432	433	420	404	362
	5	276	322	331	329	338
T. weissflogii	18	1371	1237	1178	1340	946
C. cryptica	18	116	109	114	102	106

[a]Mean of replicate determinations, using incubation periods of 2.0–2.6 h for *T. nordenskioldii* and *S. costatum* at 18°C (but 4.2–4.9 h at 5°C) and 4.5–4.6 h for *T. weissflogii* and *C. cryptica*.

[b]Culture taken without any addition to the standard growth medium.

[c]Culture taken with addition of the acetone volume (0.2 mL per 100 mL of growth medium) used for the corresponding Dimilin tests.

TABLE 2. Number of adult copepods and their reproduction surviving prolonged exposure to the Dimilin concentrations shown. All numbers represent an average of three replicates.

Days of exposure	Control	Acetone control	Dimilin (μg $\cdot L^{-1}$)				
			0.1	1.0	5.0	10.0	100
1	7.3	8.0	7.0	7.0	7.7	7.0	7.0
3	7.3	7.3	7.0	6.0	7.7	7.0	6.0
7	7.3	7.0	6.7	6.0	6.0	5.7	6.0
9	7.3	7.0	6.7	5.3	5.7	5.7	5.3
11	7.0	7.0	6.3	5.0	5.3	5.7	5.0
15	6.7	6.7	6.3	5.0	5.0	5.7	5.0
18	6.7	6.7	6.3	5.0	4.7	5.3	5.0
21	6.7	6.7[a]	6.3[a]	4.7	4.7	4.3	4.0
22	6.7[b]	6.7[a]	6.3[b]	4.7	4.7	4.0	4.0
25	6.7[b]	6.7[b]	6.7[c]	4.7	4.3[a]	4.0	4.0
31	⩾15[c,g]	6.5[b,d,f]	⩾15[c]	4.7	4.0[a]	3.7	3.7
36	⩾15[c,g]	8.0[b,d,f]	⩾15[c]	4.7	4.0	3.7	3.7
39	⩾15[c,g]	8.5[b,d,f]	⩾15[c]	4.7[a]	4.0	3.3	3.7
45	⩾15[c,g]	⩾15[b,d,g]	⩾15[c]	4.0[a,e]	3.7	3.3	3.7
51	⩾15[c,g]	⩾15[b,d,g]	⩾15[c]	4.0[a,e]	3.7	3.3	3.7
57	⩾15[c,g]	⩾15[b,d,g]	⩾15[c]	4.5[c,e]	3.3	3.0	3.7
60	⩾15[c,g]	⩾15[b,d,g]	⩾15[c]	4.5[c,e]	3.3	3.0	3.7
64	⩾15[c,g]	⩾15[b,d,g]	⩾15[c]	4.5[c,e]	3.3	3.0	3.7
71	⩾15[c,g]	⩾15[b,d,g]	⩾15[c]	2.5[c,e]	1.3	0.7	1.0

[a]One of the three replicates contained nauplii.

[b]Two of the three replicates contained nauplii.

[c]All three replicates contained nauplii.

[d]One of the three replicates never produced nauplii.

[e]All three replicates produced nauplii, but only one reached adult number ⩾15.

[f]One replicate reached adult number ⩾15; the calculated value was obtained as the mean of the other two replicates.

[g]Two replicates reached adult number ⩾15.

ticoid copepod *T. californicus* at low concentrations of the order of 1–10 μg $\cdot L^{-1}$ (ppb). Similar concentration levels of the pesticide were previously reported to reduce egg viability and injure nauplii production by the calanoid copepod *Acartia tonsa* (Tester and Costlow 1981). According to these authors, Dimilin has little effect on adult survival or fecundity (i.e. the number of eggs produced by female copepods) but strongly inhibits the development of viable juveniles from hatching of these eggs. Our observations on *T. californicus* suggest a similar mode of action of Dimilin, although we have not extended our study to details of egg development and hatching.

Surprisingly, Dimilin hardly affected photosynthesis or autotrophic growth of all the diatoms examined, even at the highest concentration (5 mg $\cdot L^{-1}$) tested, which was at least three orders of magnitude greater than that found to suppress copepod nauplii production. Such absence of effect on the chitinaceous

TABLE 3. Effects of aged[a] Dimilin stock solutions on adult copepod viability and production of oviparous females. All the numbers shown, representing total adults (TA) and oviparous females (OV), are averages of four replicates.

Period of exposure to Dimilin (d)	Dimilin ($\mu g \cdot L^{-1}$)									
	Control		1.0		2.5		5.0		10	
	OV	TA	OV	TA	OV	TA	OV	TA	OV	TA
1	2.3	6.3	2.0	6.3	2.0	6.0	1.8	6.3	2.0	6.0
2	2.0	6.0	1.5	7.0	1.0	7.0	1.3	6.8	0.8	6.8
3	2.3	5.3	1.5	6.8	1.0	7.3	1.3	6.8	1.3	7.0
5	2.3	5.8	1.3	6.8	1.5	6.5	1.5	6.8	2.5	5.8
11	2.5	5.0	2.8	5.3	2.5	5.3	3.5	4.5	2.3	5.8
15	1.5	6.3	3.0	4.8	2.5	5.3	3.0	4.3	2.8	5.3
16	1.8	5.8[b]	3.0	4.8[b]	2.8	5.0[b]	2.5	5.0[b]	2.5	5.0[b]
23	2.5	5.3[b]	2.0	5.5[b]	2.5	5.3[b]	0.8	6.0[c]	2.0	6.3[d]
32	1.8	5.0[c]	1.8	5.5[b]	2.3	5.3[b]	1.3	7.5[e]	1.3	6.5[d]
43	1.3	5.8[d]	1.0	5.8[b]	0.5	5.0[c]	1.0	4.5[e]	1.0	4.5[d]
53	0.5	7.0[d]	0.5	6.3[e]	2.0	5.5[d]	0.8	6.5[e]	0.0	5.8[d]

[a]An 18-d old acetone solution was added to seawater 10 d before copepod introduction. The acetone stock solution was stored in darkness at 0–4°C.
[b]One of the four replicate vials contained nauplii.
[c]Two of the four replicate vials contained nauplii.
[d]Three of the four replicate vials contained nauplii.
[e]All four replicate vials contained nauplii.

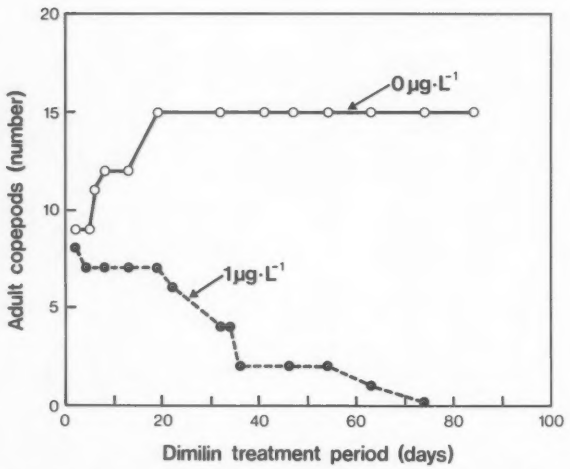

FIG. 3. Number of copepod adults found in test vials after long exposures to Dimilin. Nauplii were observed only in the control vial (0 $\mu g \cdot L^{-1}$) from the 6th day onward.

diatoms might be attributed to cell-wall impermeability of the pesticide or to a pathway of chitin biosynthesis somewhat different from that known to occur in insects and crustaceans. However, there may be no need to look for such alternative explanations, since the exact manner of Dimilin interference with chitin production in arthropods remains debatable (Meola and Mayer 1980). Recent biochemical evidence suggests that Dimilin is not inhibitory to chitin synthase isolated from a fungus or prepared from homogenates of insect pupae (Brillinger 1979; Mayer et al. 1981). It thus appears that Dimilin may not inhibit chitin biosynthesis per se but that it could act as a larvicide by interfering with one or more postsynthetic processes responsible for chitin deposition in the arthropod cuticle during ecdysis. Such an explanation might well account for the absence of injury from Dimilin to the chitinaceous diatoms, where chitin biosynthesis may occur by a pathway common to both arthropods and algae but the diatoms would not need the subsequent systems of chitin deposition into cuticles. Unfortunately, the chitin biosynthesis pathway in diatoms remains uninvestigated, and is at present believed to broadly resemble that of yeast (Duran et al. 1979) and fungi (Arroyo-Begovich and Ruiz-Herrera 1979). As described by Herth and Zugenmaier (1977) and Herth and Barthlott (1979), the chitin of diatoms is extruded extracellularly as needlelike stiff fibrils originating from special pores of the diatom valves; this description suggests little postsynthetic processing of the diatom chitin in contrast with arthropod cuticle formation.

Apart from copepods, Dimilin has also been reported to inhibit larval development of other crustaceans examined, such as crabs (Christiansen et al. 1978; Costlow 1979; Christiansen and Costlow 1982), brine shrimp (Cunningham 1976), mysids (Nimmo et al. 1979), and cladocerans (Colwell and Schaefer 1980). However, Dimilin had little or no effect on rotifers, gastropods, oligochaetes, and fish (Colwell and Schaefer 1980), as well as nitrogen-fixing blue-green algae (Wurtsbaugh and Apperson 1978). Thus, although Dimilin was designed to function as a safe insecticide, it is also injurious to valuable crustaceans and may likely harm other unexamined arthropods, because of its underlying action in inhibiting chitin deposition into larval cuticles.

Nevertheless, Dimilin appears to be the most selective insecticide designed up to now in that, apart from its deleterious effect on arthropods, it is remarkably innocuous to a wide variety of other invertebrates, some of which, like diatoms, are said to contain a sizable proportion of chitin (Muzzarelli 1977). Furthermore, Dimilin is steadily biodegraded by several nontarget organisms examined and so does not tend to bioaccumulate

in the food chain (Metcalf et al. 1975). It is also gradually degraded by sunlight, and undergoes significant hydrolytic decomposition with time in pond water, subject to pH and temperature (Schaefer and Dupras 1976). Its alleged persistence in filtered seawater was considerably weaker in our tests than that previously reported (Christiansen and Costlow 1980).

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References

ARROYO-BEGOVICH, A., AND J. RUIZ-HERRERA. 1979. Proteolytic activation and inactivation of chitin synthase from *Neurospora crassa*. J. Gen. Microbiol. 113: 339–345.

BLACKWELL, J., K. D. PARKER, AND K. M. RUDALL. 1967. Chitin fibres of the diatoms *Thalassiosira fluviatilis* and *Cyclotella cryptica*. J. Mol. Biol. 28: 383–385.

BRILLINGER, G. U. 1979. Metabolic products of microorganisms. 181. Chitin synthase from fungi, a test model for substances with insecticidal properties. Arch. Microbiol. 121: 71–74.

CHRISTIANSEN, M. E., AND J. D. COSTLOW, JR. 1980. Persistence of the insect growth regulator Dimilin in brackish water: a laboratory evaluation using larvae of an estuarine crab as indicator. Helgol. Meeresunters. 33: 327–332.

1982. Ultrastructural study of the exoskeleton of the estuarine crab *Rhithropanopeus harrisii*: effect of the insect growth regulator Dimilin (Diflubenzuron) on the formation of the larval cuticle. Mar. Biol. 66: 217–226.

CHRISTIANSEN, M. E., J. D. COSTLOW, JR., AND R. J. MONROE. 1978. Effects of the insect growth regulator Dimilin (TH 6040) on larval development of two estuarine crabs. Mar. Biol. 50: 29–36.

COLWELL, A. E., AND C. H. SCHAEFER. 1980. Diets of *Ictalurus nebulosus* and *Pomoxis nigromaculatus* altered by diflubenzuron. Can. J. Fish. Aquat. Sci. 37: 632–639.

CONOVER, S. A. M. 1975. Partitioning of nitrogen and carbon in cultures of the marine diatom *Thalassiosira fluviatilis* supplied with nitrate, ammonium or urea. Mar. Biol. 32: 231–246.

COSTLOW, J. D., JR. 1979. Effect of Dimilin on development of larvae of the stone crab *Menippe mercenaria*, and the blue crab *Callinectes sapidus*, p. 355–363. *In* W. B. Vernberg, A. Calabrese, F. P. Thurberg, and F. J. Vernberg [ed.] Marine pollution: functional responses. Academic Press, New York, NY.

CUNNINGHAM, P. A. 1976. Effects of Dimilin (TH 6040) on reproduction in the brine shrimp, *Artemia salina*. Environ. Entomol. 5: 701–706.

DEUL, D. H., B. J. DE JONG, AND J. A. M. KORTENBACH. 1978. Inhibition of chitin synthesis by two 1-(2,6-disubstituted benzoyl)-3-phenyl urea insecticides. II. Pestic. Biochem. Physiol. 8: 98–105.

DURAN, A., E. CABIB, AND B. BOWERS. 1979. Chitin synthetase distribution on the yeast plasma membrane. Science (Wash., DC) 203: 363–365.

DWELTZ, N. E., J. R. COLWIN, AND A. G. MCINNES. 1968. Studies on chitan (β-(1→4)-linked 2-acetamido-2-deoxy-D-glucan) fibers of the diatom *Thalassiosira fluviatilis* Hustedt. III. The structure of chitan from X-ray diffraction and electron microscope observations. Can. J. Chem. 46: 1513–1521.

FALK, M., D. G. SMITH, J. MCLACHLAN, AND A. G. MCINNES. 1966. Studies on chitan (β-(1→4)-linked 2-acetamido-2-deoxy-D-glucan) fibers of the diatom *Thalassiosira fluviatilis* Hustedt. II. Proton magnetic resonance, infrared, and X-ray studies. Can. J. Chem. 44: 2269–2281.

HARRISON, P. J., R. E. WATERS, AND F. J. R. TAYLOR. 1980. A broad spectrum artificial seawater medium for coastal and open ocean phytoplankton. J. Phycol. 16: 28–35.

HASLE, G. R. 1978. Some *Thalassiosira* species with one central process (Bacillariophyceae). Norw. J. Bot. 25: 77–110.

1979. *Thalassiosira decipiens* (Grun.) Jørg. (Bacillariophyceae). Bacillaria 2: 85–108.

HERTH, W. 1979. The site of β-chitin fibril formation in centric diatoms. II. The chitin-forming cytoplasmic structures. J. Ultrastruct. Res. 68: 16–27.

HERTH, W., AND W. BARTHLOTT. 1979. The site of β-chitin fibril formation in centric diatoms. I. Pores and fibril formation. J. Ultrastruct. Res. 68: 6–15.

HERTH, W., AND P. ZUGENMAIER. 1977. Ultrastructure of the chitin fibrils of the centric diatom *Cyclotella cryptica*. J. Ultrastruct. Res. 61: 230–239.

JAKOB, W. L. 1973. Developmental inhibition of mosquitoes and the housefly by urea analogues. J. Med. Entomol. 10: 452–455.

MAYER, R. T., A. C. CHEN, AND J. R. DELOACH. 1981. Chitin synthesis inhibiting insect growth regulators do not inhibit chitin synthase. Experientia 37: 337–338.

MCLACHLAN, J., AND J. S. CRAIGIE. 1966. Chitan fibres in *Cyclotella cryptica* and growth of *C. cryptica* and *Thalassiosira fluviatilis*, p. 511–517. *In* H. Barnes [ed.] Some contemporary studies in marine science. Allen and Unwin Ltd., London. 716 p.

MCLACHLAN, J., A. G. MCINNES, AND M. FALK. 1965. Studies on the chitan (chitin: poly-*N*-acetylglucosamine) fibers of the diatom *Thalassiosira fluviatilis* Hustedt. I. Production and isolation of chitan fibers. Can. J. Bot. 43: 707–713.

MEOLA, S. M., AND R. T. MAYER. 1980. Inhibition of cellular proliferation of imaginal epidermal cells by diflubenzuron in pupae of the stable fly. Science (Wash., DC) 207: 985–987.

METCALF, R. L., P.-Y. LU, AND S. BOWLUS. 1975. Degradation and environmental fate of 1-(2,6-difluorobenzoyl)-3-(4-chlorophenyl) urea. J. Agric. Food Chem. 23: 359–364.

MULDER, R., AND M. J. GIJSWIJT. 1973. The laboratory evaluation of two promising new insecticides which interfere with cuticle deposition. Pestic. Sci. 4: 737–745.

MUZZARELLI, R. A. A. 1977. Chitin. Pergamon Press, Oxford. 309 p.

NIMMO, D. R., T. L. HAMAKER, J. C. MOORE, AND C. A. SOMMERS. 1979. Effect of diflubenzuron on an estuarine crustacean. Bull. Environ. Contam. Toxicol. 22: 767–770.

POST, L. C., B. J. DE JONG, AND W. R. VINCENT. 1974. 1-(2,6-disubstituted benzoyl)-3-phenyl urea insecticides: inhibitors of chitin synthesis. Pestic. Biochem. Physiol. 4: 473–483.

SCHAEFER, C. H., AND E. F. DUPRAS, JR. 1976. Factors affecting the stability of Dimilin in water and the persistence of Dimilin in field waters. J. Agric. Food Chem. 24: 733–739.

STRICKLAND, J. D. H., AND T. R. PARSONS. 1972. A practical handbook of seawater analysis. Bull. Fish. Res. Board Can. 167 (Second Edition): 310 p.

TESTER, P. A., AND J. D. COSTLOW, JR. 1981. Effect of insect growth regulator Dimilin (TH 6040) on fecundity and egg viability of the marine copepod *Acartia tonsa*. Mar. Ecol. Prog. Ser. 5: 297–302.

WURTSBAUGH, W. A., AND C. S. APPERSON. 1978. Effects of mosquito control insecticides on nitrogen fixation and growth of blue-green algae in natural plankton associations. Bull. Environ. Contam. Toxicol. 19: 641–647.

Evidence of Contaminant Loading to Lake Ontario from the Niagara River

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The hypothesis that the concentrations of pollutants are the same throughout the length of the Niagara River, against the alternative that they are higher in the lower river, was examined using three sets of data: (1) Ontario Ministry of Environment's (MOE) results for organic substances in raw drinking water (1978–84); (2) MOE transect data for metals in water (1981); (3) Environment Canada data on organic substances in large-volume water samples and suspended sediments (1981). The designs of the studies dictated different statistical procedures for each set, with nonparametric procedures based on ranking being used for sets 1 and 3, and normal theory methods for set 2. Further, both univariate and multivariate procedures were used for set 3. Despite the considerable number of nondetections in these data, it was still possible to make quantitative comparative statements, with the degree of uncertainty providing the quantitative component. In each study, a number of substances were shown to be higher in the lower river than in the upper river, and by examining all substances of set 3 simultaneously, locations in the lower river were shown to be similar to each other as were locations in the upper river.

L'alternative hypothétique suivante : les concentrations de matières polluantes sont semblables dans tout le cours de la rivière Niagara, ou les concentrations sont plus élevées dans le cours inférieur, a été évaluée à l'aide de trois séries de données : (1) les résultats de quantification des substances organiques dans l'eau potable non traitée recueillis par le ministère ontarien de l'Environnement (MOE) de 1978 à 1984; (2) les données par coupe transversale sur les métaux dissous dans l'eau, recueillies par le MOE en 1981; (3) les données d'Environnement Canada sur les substances organiques contenues dans de grands volumes d'eau échantillonnés et dans les sédiments en suspension (1981). La conception des études a déterminé le genre d'analyse statistique appliquée à chaque série de données : des méthodes non paramétriques basées sur le classement ont été utilisées pour les séries 1 et 3 et la méthode basée sur la théorie normale, pour la série 2. De plus, la série 3 a fait l'objet d'analyses unifactorielle et multifactorielle. Malgré le nombre élevé de non-détections dans ces données, on a pu faire des comparaisons quantitatives où le degré d'incertitude représentait la composante quantitative. Dans chaque étude, la concentration d'un certain nombre de substances s'est révélée plus élevée dans le cours inférieur que dans le cours supérieur de la rivière. L'examen simultané de toutes les substances de la série 3 a démontré que les emplacements dans le cours inférieur étaient semblables, de même que ceux dans le cours supérieur.

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Numerous studies have demonstrated that the Niagara River is a major source of the pollutants that enter Lake Ontario. The problem, summarized in Allan et al. (1983), is that the Niagara River, which is the only outflow for Lake Erie and the major tributary to Lake Ontario (83% of the total water input), receives chemical substances from waste dumps and municipal and industrial outfalls, primarily from the concentration of chemical industries on the New York State side of the river.

Although it is clear that chemicals are entering Lake Ontario from the Niagara River, it is difficult to make quantitative comparative statements about the amounts of many organic substances entering the lake because environmental concentrations are too close to the analytical limits of detection. We address the question, are the concentrations of substances in water and sediments higher in the lower river than in the upper river? Three very different sets of data are used, and thus different statistical methods are applied. The results show that

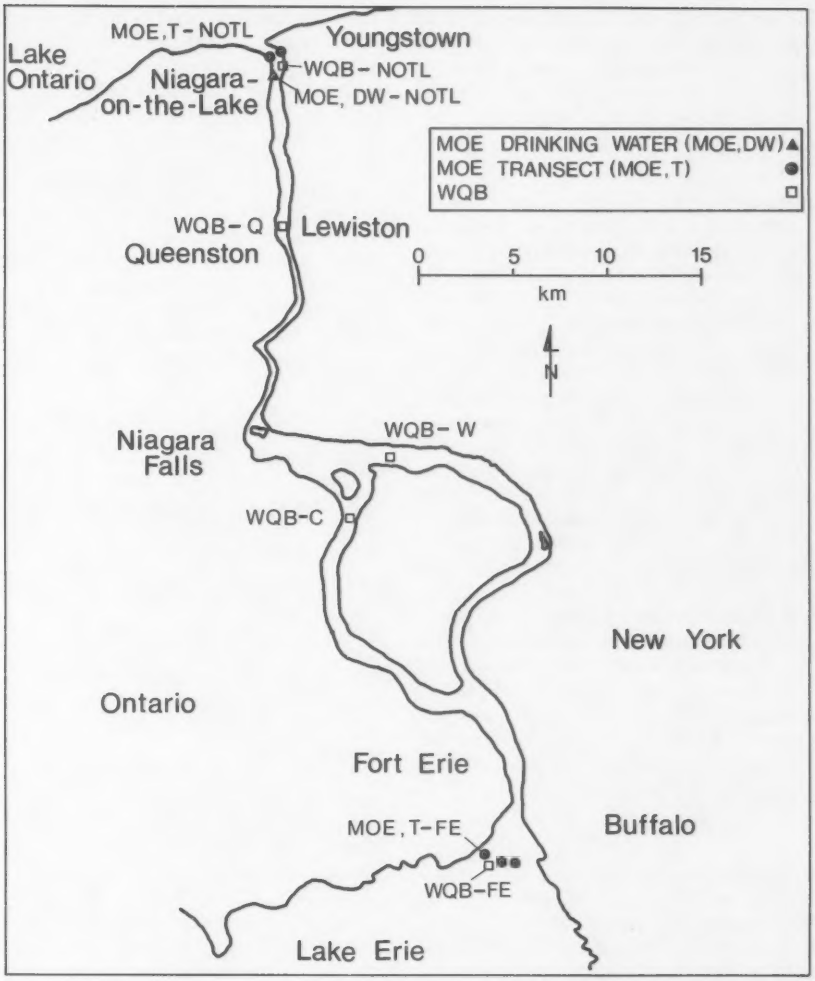

FIG. 1. Location of sampling stations on the Niagara River for all three studies.

comparative statements can be made quantitative, where the quantification is given by the degree of uncertainty (usually the significance level) attached to the statement, and that this can be done even when a substantial number of nondetections occur in the data.

Data and Methods

Data Sets

The following three data sets, collected under different sampling strategies, were available for analysis:

(1) From the Ontario Ministry of the Environment (MOE) monitoring program for volatile organics in raw drinking water, the results of the analyses for 24 of the substances classified as priority pollutants by the US EPA, 22 other organics, and 23 pesticides in water samples collected at Fort Erie (FE) and Niagara-on-the-Lake (NOTL) between August 1978 and January 1984.

(2) From the MOE transect study, the concentrations of nine metals in samples from the three stations on the FE transect which are representative of Lake Erie and all five stations on the NOTL transect, where sampling was done on three successive days in the months of June, August, and September of 1981.

(3) The results of the analysis for 28 chlorinated organic substances in large-volume water samples and in suspended sediments obtained by the Water Quality Branch (WQB), Environment Canada, at five stations (Fig. 1): FE, Chippawa (C), Wheatfield (W), Queenston (Q), and NOTL, with one or two samples per station once during May, July, and October–November of 1981.

In contrast with the last two sets of data, drinking water was collected over a number of years, at various times during the year, and with different frequencies for the two stations. Not all substances were reported for each sample, and the result reported was often a nondetection. The metal data was mostly quantitative, with some metals not reported for some samples and some results removed because they had been flagged as being of questionable quality. The stations used within a transect are not expected to show spatial variability. In the WQB study, results were reported for all substances in all samples, but these included numerous nondetections. Since data for only two

locations were used from the MOE data sets, the objective is to determine if there are differences in the levels of a substance between the locations. The data from five locations in the WQB study permit not only the analogous comparison of levels, but also the analysis for trend due to position down the river.

Analytical Methods

The analytical methods used for these three data sets have been independently reviewed and accepted by the Canada – United States Niagara River Toxics Committee (Data Quality Subcommittee 1984). In the MOE studies, pesticides and PCBs were analyzed using gas chromatography with an electron capture detector to detection limits of 0.001–0.005 μg/L for pesticides and 0.02 μg/L for PCBs. GC/MS purgeables were analyzed to a detection limit of 0.1 μg/L and the GC/MS extractable analysis was qualitative only. Metals were determined using either flame atomic absorption or inductively coupled plasma spectrometry. Total tetrachloro dibenzo-*p*-dioxins were analyzed by GC/MS with a detection limit of 0.25 ng/L prior to May 1983, and after May 1983 to a detection limit for each individual isomer of tetrachloro dibenzo-*p*-dioxins of 0.01 ng/L (MOE 1984). The method used for the WQB large-volume water samples (McCrea 1982) and suspended sediments (Kuntz and Warry 1983) consists of collection of a sample over an approximate 2-d period during which the suspended sediment sample is separated by means of a Westfalia continuous-flow separator and a 200-L sample of the effluent is collected for the water sample. The water samples are extracted with dichloromethane, the sediment samples pressure-filtered, dried, and ground, and then both analyzed at the WQB Organic Analysis Laboratory according to methods described in the *Analytical Methods Manual* (Environment Canada 1979).

Statistical Methods

For the drinking water data, the comparison of the level of a substance at NOTL and the level at FE has been based on samples taken at the two stations at about the same time (usually the same day). The sign test (e.g. Lehmann and D'Abrera 1975) was applied if enough matched sampling dates were available for a substance. The sign for the pair was determined by ranking one station relative to the level of the other. Let (x_{i1}, x_{i2}) be the results for the analysis of the substance under study at locations 1 and 2 on day i for $i = 1, 2, \ldots, n$. Then

$d_i = +$ if either

(i) x_{i1} and x_{i2} are measurable and $x_{i1} > x_{i2}$,

(ii) x_{i1} is measurable and x_{i2} is recorded as trace (Tr) or not detected (ND), or

(iii) x_{i1} = Tr and x_{i2} = ND;

$d_i = -$ if either

(i) x_{i1} and x_{i2} are measurable and $x_{i1} < x_{i2}$,

(ii) x_{i1} = Tr or x_{i1} = ND and x_{i2} is measurable, or

(iii) x_{i1} = ND and x_{i2} = Tr;

and $d_i = 0$ if either

(i) x_{i1} and x_{i2} are measurable and $x_{i1} = x_{i2}$,

(ii) $x_{i1} = x_{i2}$ = Tr, or

(iii) $x_{i1} = x_{i2}$ = ND.

Let N_+, N_-, and N_0 be the number of d_i that are $+$, $-$, and 0, respectively. The objective is to test the hypothesis of no difference between the concentrations of a substance at the two stations against the alternative that the concentrations are higher at NOTL. In the presence of ties, $N_0 \neq 0$, two statistics are available (Lehmann and D'Abrera 1975). The most powerful test is based on the statistic N_+ (Putter 1955), which is obtained by conditioning on the observed number of ties, $N_0 = n_0$, and is equivalent to omitting the ties. A statistic that includes the ties is $N_+ + 1/2N_0$, but the distribution of this contains the unknown parameter, p_0, which is the probability of obtaining a tie. N_+ has a binomial distribution with parameters $n - n_0$ and $p = 0.5$, and the exact significance level associated with an observed value of N_+ can be calculated. The customary procedure for obtaining the significance level for $N_+ + 1/2N_0$ is to use the binomial distribution with parameters n and $p = 0.5$ (Putter 1955). The asymptotic test is the same for both statistics and is obtained from

$$Z = (2N_+ - n + N_0)/(n - N_0)^{1/2}$$

which has approximately a $N)0, 1)$ distribution.

The analysis of the transect data for metals at NOTL and FE was based on a partially nested model (Scheffé 1959). Days are nested within months but months and stations are crossed factors. The model is given by

$$x_{ijkl} = \mu + m_i + d_{j(i)} + s_k + (sm)_{ik} + (sd)_{jk(i)} + e_{ijkl}$$

where m_i is the effect of month i, $d_{j(i)}$ is the effect of day j within month i, s_k is the station effect for station k, $(sm)_{ik}$ is the interaction between month i and station k, and $(sd)_{jk(i)}$ is the interaction between station k and day j within month i. The subscript l refers to replicates and the e_{ijkl} are assumed independent $N(0, \sigma^2)$.

The design must either be balanced (equal number of replicates) or have proportional frequencies (Kendall and Stuart 1968) if the sums of squares are to be calculated in the usual way (Bennett and Franklin 1954) as sums of the squared differences between means. Because of the unequal number of replicates for the two stations, the sums of squares were obtained using "dummy" variables and regression methods (Draper and Smith 1981). The residual sum of squares obtained when all main effects and interactions are included in the model provides the error sum of squares for testing the significance of effects. The sums of squares of main effects and interactions are obtained individually as the difference between the residual sum of squares, with the parameters corresponding to the particular main effect or interaction omitted, and the residual sum of squares for the full model. Once the sums of squares are calculated the analysis of variance can be conducted in the usual way.

In the presence of significant interactions, the effects of a corresponding factor must be examined at each level of the other factor. The *t*-test was used to compare stations for the individual days. Let $\bar{x}_{ijk}$ and s^2_{ijk} be the mean and the variance from the n_{ijk} samples taken at station k on day j of month i, and s^2 be the within-day variance obtained by pooling a number of s^2_{ijk}. Then the *t*-test for day j, with $k = 1$ for NOTL and $k = 2$ for FE, is given by

$$t = (\bar{x}_{ij1} - \bar{x}_{ij2})/s \sqrt{\frac{1}{n_{ij1}} + \frac{1}{n_{ij2}}}$$

where t follows a t-distribution with degrees of freedom equal to that of s. For the days exhibiting higher variation, the test due to Welch (Brownlee 1965) for the equality of means in samples with different variances was applied. The test statistic is

$$t' = (\bar{x}_{ij1} - x_{ij2})/\sqrt{A + B}$$

where $A = s^2_{ij1}/n_{ij1}$, $B = s^2_{ij2}/n_{ij2}$, and t' has approximately a t-distribution with degrees of freedom

$$f' = \frac{(A + B)^2}{A^2/(n_{ij1} - 1) + B^2/(n_{ij2} - 1)}.$$

The statistical analysis of the WQB data attempts to answer the following questions: (1) Are there overall differences between the levels of the chlorinated organics at the five sampling stations? (2) If the answer is yes in (1), then, is there a consistent pattern for the differences? (3) What is the direction of this pattern? (4) What can be said about the behaviour of individual substances? The analysis was performed using ranks that were assigned as follows: let $x_1, x_2, \ldots, x_n$ be the concentrations of a specific substance at a given data in samples from locations $1, 2, \ldots, n$, where x is the mean for those dates when two samples were taken. The smallest x_1 is replaced by 1, the second smallest by 2, ..., and the largest by n. In the case of ties, the mid-rank is used. Rank one is given when x is below the detection limit, and the mid-rank is used when there is more than one x below the detection limit. Thus, the data tables were replaced by tables of the ranks prior to the analysis.

Let R_{ij} be the rank of the ith substance ($i = 1, 2, \ldots, l$) at the jth station ($j = 1, 2, \ldots, n$) where l is the number of substances. The squared Euclidean distance, ED(j, j'), between locations j and j' is

$$\mathrm{ED}(j, j') = \sum_{i=1}^{l} (R_{ij} - R_{ij'})^2.$$

It can be shown that under the hypothesis of no differences between the stations and independence between the substances, the mean of ED(j, j') is

$$M = \frac{ln(n + 1)}{6} - \frac{\sum_{i=1}^{l} \sum_{s=1}^{g} d_{is}(d^2_{is} - 1)}{6(n - 1)}$$

where for the ith substance d_{i1} gives the number of locations with the common rank $(d_{i1} + 1)/2$, d_{i2} gives the number of locations with the common rank $d_{i1} + (d_{i2} + 1)/2$, and so on. When there are no tied observations for the ith substance, all $d_{is} = 1$. The expression for the variance is

$$\sigma^2 = \frac{2}{n - 1} \sum_{i=1}^{l} \left\{ \sum_{s} U^4_{is} - 2(n + 1) \sum_{s} U^3_{is} + 3 \left[\frac{n(n + 1)(2n + 1)}{6} - \frac{\sum_{s} d_{is}(d^2_{is} - 1)}{12} \right]^2 \right\} - M^2$$

where $U_{is} = d_{i1} + d_{i2} + \ldots + d_{is} + d_{is+1}/2$. In the case where $d_{is} = 1$ for all i and s, the above expressions reduce to

$$M = \frac{ln(n + 1)}{6}$$

and

$$\sigma^2 = ln(n + 1)(7n^2 - 5n - 18)/180.$$

The standardized Euclidean distance is

$$\mathrm{SED}(j, j') = \{\mathrm{ED}(j, j') - M\}/\sigma.$$

Under the assumptions that the locations are equally similar to each other, the values of SED(j, j') will be close to zero. Large negative (positive) values of SED(j, j') indicate that the locations are more similar (less similar) than that expected under equal similarity between the locations. The values of SED(j, j') are used to form clusters using the complete-linkage clustering method (Anderberg 1973).

The differences between locations for each individual substance are examined as follows. Let x_{ij} be the concentration of the substance of interest at the ith time ($i = 1, 2, \ldots, a$) and the jth location ($j = 1, 2, \ldots, n$) and let R_{ij} be the rank of x_{ij} in the joint ranking of $x_{i1}, x_{i2}, \ldots, x_{in}$. The Page test (Hollander and Wolfe 1973, p. 147) is designed to test for an increasing trend. The test statistic is

$$S_1 = \sum_{j=1}^{a} jR_j$$

where

$$R_j = \sum_{i=1}^{n} R_{ij}.$$

Critical values of S_1 are given for small values of a and n in table A(16) in Hollander and Wolfe (1973). For large n the distribution of S_1 is normal with mean $na(a + 1)^2/4$ and variance $n(a^3 - a)^2/144(a - 1)$, and hence the normal distribution could be used to calculate the significance level associated with an observed value of S_1.

Results

MOE Drinking Water Data

The hypothesis of equal concentration at NOTL and FE versus the alternative that the concentration is higher at NOTL than at FE was tested for 15 of the priority pollutants, eight of the organic substances, and two of the pesticides using the one-sided sign test (Table 1). The test was performed only if the number of pairs, n_d, exceeded three, since even if the NOTL level is higher than the FE level for each pair when the number of pairs of samples equals three, the significance level is 0.13. Many of the 69 substances were either never detected or detected no more than three times (Tables 2 and 3). Of the 25 substances tested, the following four were significant at the 1% level: chloroform, trichloroethylene, tetrahydrofuran, and *p*-chlorotrifluorotoluene (Table 1). In addition, two more were significant at the 5% level (tetrachloroethylene and A-BHC), and three more at the 10% level (benzene, 2,4,6-trichlorophenol, and chlorotoluene) for a total of four substances significant at the 1% level, six at the 5% level, and nine at the 10% level. These conclusions hold for the tests based on both N_+ and $N_+ + 1/2N_0$, and over all the substances, the two significance levels were very similar. Note that the significance level for dimethyldisulphide is high, since all of the six samples showed higher levels at FE than at NOTL and the one-sided test conducted here gives the probability of obtaining a number of positive signs at least as large as that observed, namely $r = 0$. That is, the only evidence included, against the hypothesis of equal concentration, is evidence in the direction of NOTL > FE.

A cumulative sum plot can be used to examine the constancy of the difference between the stations over time. For a particular substance, let R_j = the number of times the level of the substance at NOTL exceeds that at FE for the first j dates. The plot of the cumulative sum of the difference between R and its expected value $E(R)$ is shown for chloroform in Fig. 2. For the

TABLE 1. Test of the hypothesis of equal concentration at NOTL and FE for substances in raw drinking water based on matched sampling dates, MOE data.

Class	Compound	n_+	$n - n_0$	Pc	n_0	$n_+ + 1/2n_0$	P
Priority pollutants	Methylene chloride	2	4	0.69	0	0	Pc
	Chloroform	30	43	0.007	10	35	Pc
	1,1,1-Trichloroethane	4	6	0.34	3	5.5	0.26
	Dichlorobromomethane	10	16	0.23	0	10	Pc
	Trichloroethylene	15	18	0.004	2	16	0.006
	Chlorodibromomethane	11	17	0.17	0	11	Pc
	Benzene	16	24	0.08	12	22	Pc
	Tetrachloroethylene	8	10	0.05	3	9.5	0.05
	Toluene	10	24	0.85	13	16.5	Pc
	m-Xylene	5	9	0.50	4	7	0.50
	o,p-Xylene	8	13	0.29	6	11	0.32
	Hexane	9	13	0.13	4	11	0.17
	Hexanal	5	9	0.50	4	7	0.50
	Tetrahydrofuran	23	23	0	0	23	Pc
	p-Chlorotrifluorotoluene	10	10	0.001	0	10	Pc
Organics	Dimethyldisulphide	0	6	1	1	0.5	0.99
	Diethyl ether	5	7	0.23	0	5	Pc
	2,4,6-Trichlorophenol	4	4	0.06	0	4	Pc
	Carbon disulphide	3	4	0.31	1	3.5	0.19
	Methyl ethyl ketone	4	7	0.50	2	5	0.50
	Chlorotoluene	4	4	0.06	0	4	Pc
	Acetone	5	9	0.50	11	10.5	0.42
	Hydrocarbons	8	16	0.60	10	13	0.55
Pesticides	A-BHC	18	26	0.04	6	21	Pc
	G-BHC	6	9	0.25	2	7	0.27

NOTE: n_+ = number of times the level was higher at NOTL than at FE. n_0 = number of times the levels were the same at NOTL and FE. n = total number of pairs. Pc = one-sided observed significance level, based on n_+. It is the exact significance level if $n - n_0 \leq 20$ or the normal approximation if $n - n_0 > 20$. P = one-sided observed significance level based on $n_+ + 1/2n_0$. It is calculated from the binomial distribution with parameters n and $1/2$ if $n \leq 20$ or the normal approximation if $n > 20$. To calculate the significance level from noninteger values and the binomial distribution, the smallest integer $> n_+ + 1/2n_0$ was used. Pc appearing in this column indicates that the significance level equals that based on n_+.

TABLE 2. Compounds not detected at either NOTL or FE on matched sampling dates, MOE drinking water data.

Priority pollutants	Organics	Pesticides
Dichloroiodomethane	Total trichlorophenols	Aldrin
1,1,4-Trichlorobutadiene	2,3,4,5-Trichlorophenol	DMDT–methoxychlor
Bromoform	3,4,6-Trichlorophenol	Endosulphan II
	Dichloropropylene	Endosulphan sulphate
		Heptachlor
		Mirex
		Oxychlordane
		o,p-DDT
		p,p-DDD
		p,p-DDE
		Total tetrachlorodioxins

*i*th sampling date this is

$$\sum_{j=1}^{i} (R_j - E(R_j)) = \sum_{j=1}^{i} (R_j - j/2)$$

and this has been plotted against the date, to the nearest half-month, which corresponds to i. If there is no difference between the chloroform concentration at NOTL and FE, the points in Fig. 2 should fluctuate around zero. On the contrary, the points are always above zero and continue to increase throughout most of the sampling period. This indicates that the concentration at NOTL remains above that at FE, except possibly from about July 1981 to the end of 1982.

MOE Transect Data

The analysis of variance was performed for four metals (Table 4). The interactions between station and day within month and between station and month were significantly differ-

TABLE 3. Compounds detected not more than three times at either NOTL or FE on matched sampling dates, MOE drinking water data.

Priority pollutants			Organics			Pesticides		
	Occurrences			Occurrences			Occurrences	
Compound	NOTL	FE	Compound	NOTL	FE	Compound	NOTL	FE
1,1-Dichloroethane	1	0	2,3,5-Trichlorophenol	2	0	A-chlordane	1	0
1,2,-Dichloroethane	1	0	2,3,6-Trichlorophenol	1	0	G-chlordane	1	0
1,2-Dichloropropane	1	0	Dichloroethylene	1	0	Endosulphan I	1	0
Chlorobenzene	1	0	Dichlorobenzene	3	0	Endrin	1	0
Ethyl benzene	2	0	Styrene	1	0	*p,p*-DDT	0	1
Carbon tetrachloride	2	1	Heptanone	2	0	Hexachlorobenzene	2	0
			Benzaldehyde	0	1	Total PCB	2	0
			Methyl furan	0	3	Dieldrin	0	2
			Trichlorotrifluoroethane	0	2	Heptachlor expoxide	0	2
			Butanol	2	0	B-BHC	3	0

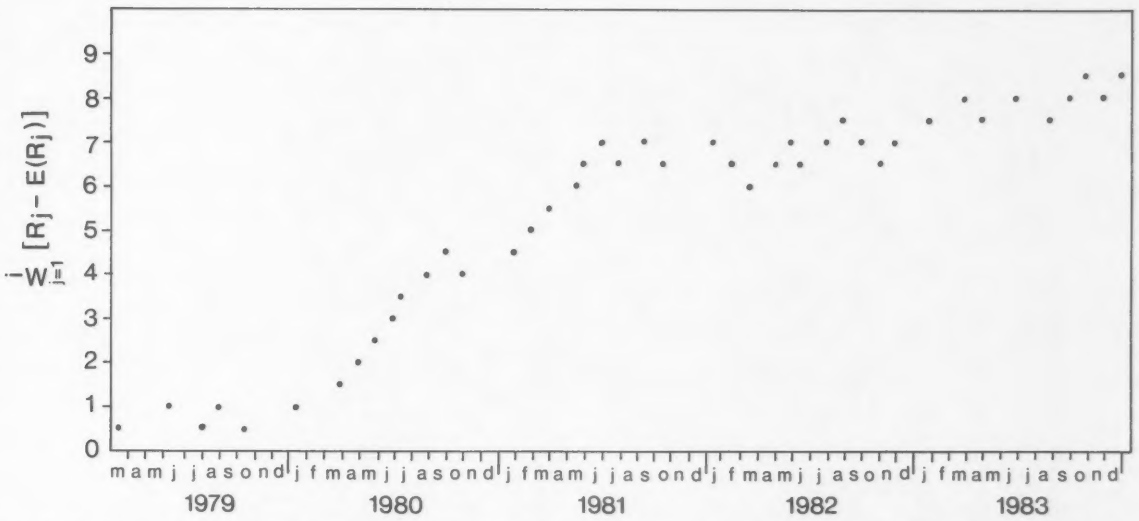

FIG. 2. Cumulative sum of the difference between the number of +'s up to sample *i* and its expected value calculated for MOE chloroform data and plotted against the date of sample *i*.

TABLE 4. Analysis of variance based on partially nested model for four of the metals in the MOE transect study (df = degrees of freedom, MS = sum of squares, *F* = ratio of mean squares, *P* = observed significance level).

DF (replicates, total) source	df	Iron (53,70)			Aluminum (53,70)			Zinc (52,69)			Copper (51,68)		
		MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Between months	2	0.00495	2.86	0.06	0.00021	1.30	0.272	6.47×10^{-6}	0.20	0.975	1.23×10^{-5}	0.09	0.996
Between days within months	6	0.00290	1.68	0.14	0.00452	9.35	<0.001	7.94×10^{-5}	2.41	0.098	0.00078	5.87	0.005
Between stations	1	0.00306	1.77	0.19	0.00017	0.18	0.677	1.33×10^{-7}	0.00	0.905	7.01×10^{-6}	0.05	0.805
Station × month interaction	2	0.00809	4.68	0.01	0.00072	4.48	0.001	1.74×10^{-6}	0.05	0.999	2.03×10^{-5}	0.15	0.987
Station × day within month interaction	6	0.00470	2.72	0.02	0.00594	12.29	<0.001	8.66×10^{-5}	2.63	0.080	0.00066	4.90	0.011
Between replicates		0.00173			0.00097			3.29×10^{-5}			0.00013		
Total (corrected)		0.00610			0.00324			4.02×10^{-5}			0.00015		

TABLE 5. Summary of analyses of MOE transect metal data where units of concentration are

		Iron						
		Mean concentration		Significance level			Mean concentration	
Month	Day	NOTL	FE	s^2 9 days	s^2 7 days	$s_1^2 \neq s_2^2$	NOTL	FE
6	11	0.310	0.129	<0.0001	<0.0001		0.236	0.13
	12	0.176	0.096	0.005	0.001		0.160	0.10
	13	0.160	0.054	0.0005	—	0.02	0.152	0.07
8	5	0.067	0.088	0.75	0.81		0.084	0.09
	6	0.112	0.052	0.03	0.008		0.107	0.06
	7	0.110	0.125	0.80	—	0.65	0.111	0.05
9	23	0.222	0.076	<0.0001	<0.0001		0.206	0.12
	24	0.170	0.075	0.0015	0.0002		0.180	0.10
	25	0.096	0.056	0.09	0.05		0.096	0.08

		Copper					
		Mean concentration		Significance level			
Month	Day	NOTL	FE	s^2 9 days	s^2 7 days	$s_1^2 \neq s_2^2$	NO
6	11	0.011	0.018	0.80	1.00		0.
	12	0.020	0.011	0.15	—	0.25	0.
	13	0.001	0.011	0.84	1.00		0.
8	5	0.003	0.007	0.67	0.89		0.
	6	0.008	0.003	0.30	0.07		0.
	7	0.007	0.007	0.54	0.57		0.
9	23	0.003	0.006	0.61	0.78		0.
	24	0.005	0.032	1.00	—	0.82	0.
	25	0.006	0.004	0.40	0.73		0.

ation are mg/L.

Aluminum				Zinc				
Mean entration	Significance level			Mean concentration		Significance level		
FE	s^2 9 days	s^2 7 days	$s_1^2 \neq s_2^2$	NOTL	FE	s^2 9 days	s^2 8 days	$s_1^2 \neq s_2^2$
0.133	<0.0001	<0.0001		0.014	0.013	0.41	0.36	
0.100	0.005	0.0006		0.012	0.007	0.13	0.03	
0.075	0.008	—	0.04	0.002	0.005	0.73	0.84	
0.095	0.69	0.74		0.005	0.006	0.62	0.68	
0.069	0.05	0.015		0.010	0.002	0.03	—	0.18
0.057	0.01	0.002		0.005	0.008	0.72	0.83	
0.124	0.001	—	0.17	0.004	0.003	0.40	0.35	
0.102	0.0007	<0.0001		0.005	0.004	0.37	0.30	
0.086	0.34	0.29		0.003	0.003	0.45	0.44	

Mean concentration							
Nickel		Chromium		Cadmium		Lead	
NOTL	FE	NOTL	FE	NOTL	FE	NOTL	FE
0.003	0.004	0.002	0.002	0.002	0.002	0.010	0.004
0.002	0.002			0.002	0.005		
0.005	0.002	0.004	0.026	0.003	0.002		
0.003	0.006	0.003	0.002	0.003	0.002	0.005	0.005
0.003	0.003	0.003	0.002	0.002	0.003	0.003	0.005
0.002	0.003	0.002	0.002				
0.002	0.003	0.004	0.009				
0.005	0.002	0.004	0.002	0.003	0.007		
0.002	0.004	0.003	0.002	0.003	0.003	0.005	0.006

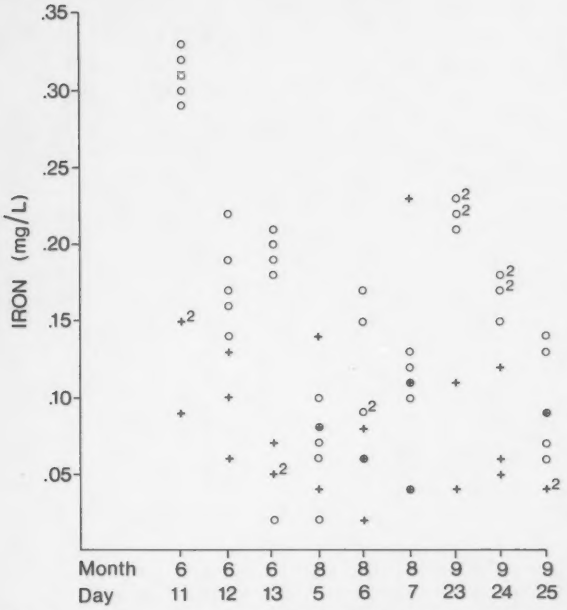

FIG. 3. Iron concentration by day for NOTL (○) and FE (+) stations, MOE transect data. The numbers beside the symbols indicate the number of stations with the same concentration within the resolution of the plot.

ent from zero at the 0.02 level or lower for iron and aluminum, and between station and day within month at the 0.01 level for copper. No effects were significantly different from zero for zinc. The analysis could end here for zinc. Due to the significant interactions, the station effect for iron, aluminum, and copper had to be evaluated for each day (Table 5).

Logarithms are often used in the analysis of environmental data. Since replicates were available, the relationship between the variance and mean was examined by plots of s^2_{ijk} versus $\bar{x}_{ijk}$. The increase in s_{ijk} with $\bar{x}_{ijk}$, which motivates the transformation to logarithms of the concentration, was definitely not present for this data set. The major feature was one or two larger within-day variances. The residual sum of squares in the present analysis of variance is the between replicate sum of squares which is given by

$$\sum_i \sum_j \sum_k \sum_l (x_{ijkl} - \bar{x}_{ijk})^2 = \sum_i \sum_j \sum_k (n_{ijk} - 1)s^2_{ijk}.$$

The residual mean square provides the estimate of the variance to be used to test equality of station means separately for each day. Where larger values of s^2_{ijk} were indicated from the plots, the tests of station effect were also evaluated with those excluded. Thus, in Table 5, there are three columns of significance levels for each metal. The first two correspond to the result of a t-test using the estimate of the variance given by the between-replicates mean square (Table 4) and by the between-replicates mean square where days with larger variance at either NOTL or FE have been eliminated. These t-tests are based on degrees of freedom in the range 39–53. For the days exhibiting larger variation, the Welch test was applied and these results appear as the third column in each set of columns of significance levels. Significance levels have been quoted for zinc, since weak evidence of interactions was present. Because the concentrations show very little variability and were reported to only one significant digit, it was decided that the analytical method used for the other five metals was not sensitive enough to detect differences in concentration. Thus, the above-described tests were not applied. Mercury was completely omitted because it was analysed on 3 d only.

Of the metals, only iron and aluminum had concentrations reported to two or three significant digits. Further, with the exception of zinc on August 6, only iron and aluminum showed significant differences between NOTL and FE. For iron, the concentrations at the two stations were more different in June and September than in August and for aluminum in June (e.g. Fig. 3).

WQB Data: Differences between Locations

Figures 4A and 4B showed that NOTL and Q are similar to each other (SED(Q, NOTL) < 0) and different from the rest of the locations. The locations FE, C, and W always form a group with SED(j, j') negative, except for the chlorinated organics in the aqueous phase in July where location W was linked with locations FE and C at a positive value for SED(j, j').

To determine the pattern of the differences between the locations, the substances were divided into three groups (PCBs, pesticides, and chlorinated aromatics). Under the assumption that there are no differences between locations, it is expected that the mean rank for each group should be close to three. The plots of mean ranks (Fig. 5A and 5B) show that (1) the pesticides are not good for differentiating between locations and (2) NOTL and Q are similar to each other and different from FE, C, and W. In fact, the mean ranks for NOTL and Q are consistently greater than three for the chlorinated aromatics and PCBs in both the suspended and the aqueous phase. This indicates an overall increase in the concentrations of PCBs and the chlorinated aromatics in the lower Niagara River as compared with the upper part of the river.

WQB Data: Trend Analysis for Individual Substances

Table 6 gives the value of S_1 for each substance, for the suspended sediments, aqueous phase, and for combined data. The results show the existence of a significant increase in the concentration of 16 substances in either the suspended phase or in the aqueous phase, while for six substances most of the data were below the detection level and hence the analysis was not performed. This shows that eight substances had no increasing trend from FE to NOTL.

Discussion

Each study provides evidence that some substances are present in either water or suspended sediments at levels that are higher in the lower river than in the upper river. The WQB results, which were obtained from large-volume samples, showed many more significant results. Because the data sets are so different, it is difficult to draw a general conclusion more specific than that some differences are detectable using the available data. Not only are the sampling strategies and analytical methods different, but also the substances analyzed. More differences might be detected if adequately sensitive methods of analysis were available. This was observed (1) in comparing the number of organic substances significantly different using the more sensitive WQB methods relative to those of MOE and (2) in the analysis of MOE transect data, where more differences were detectable for the two metals that had the highest concentrations.

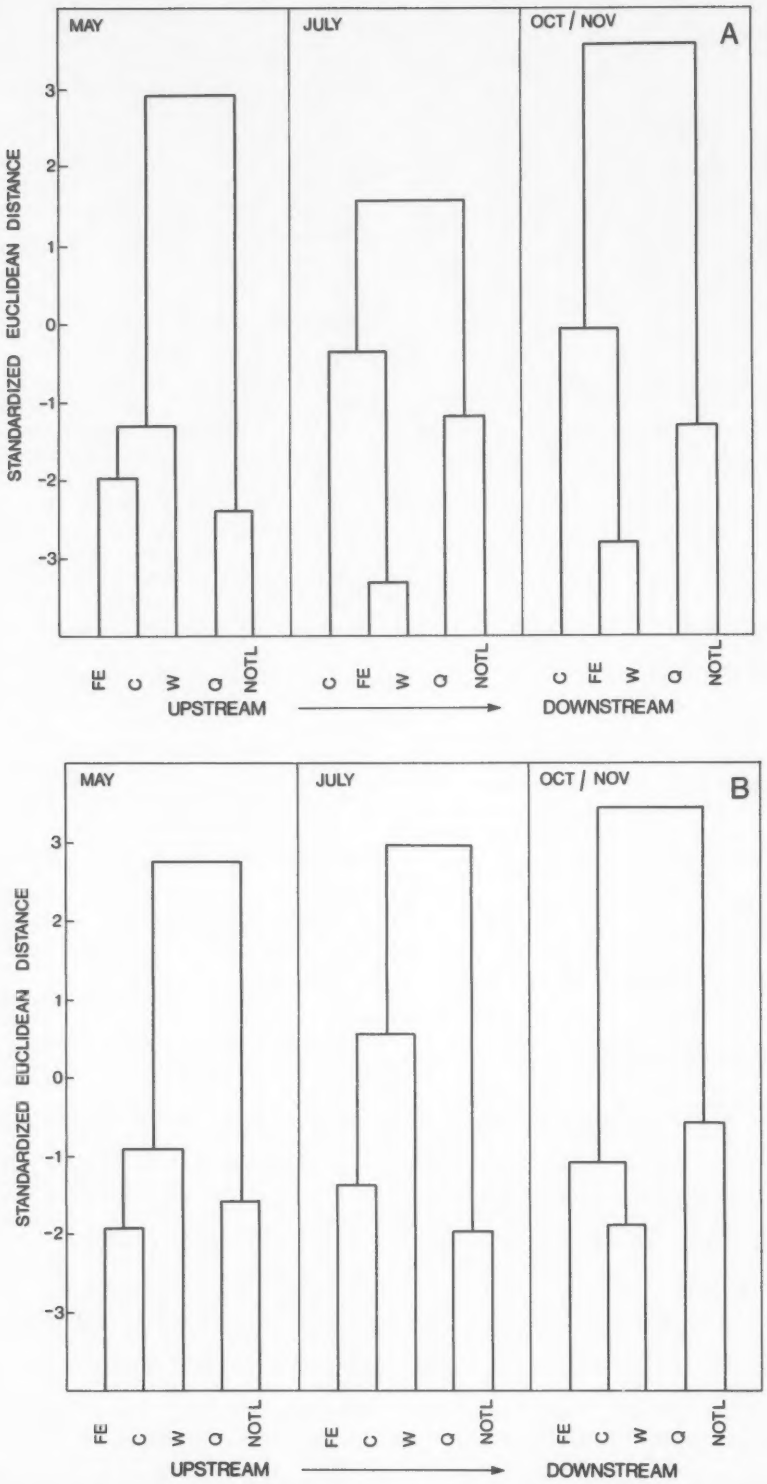

FIG. 4. Complete linkage clustering using the standardized Euclidean distance for (A) WQB suspended sediment data and (B) WQB aqueous phase data.

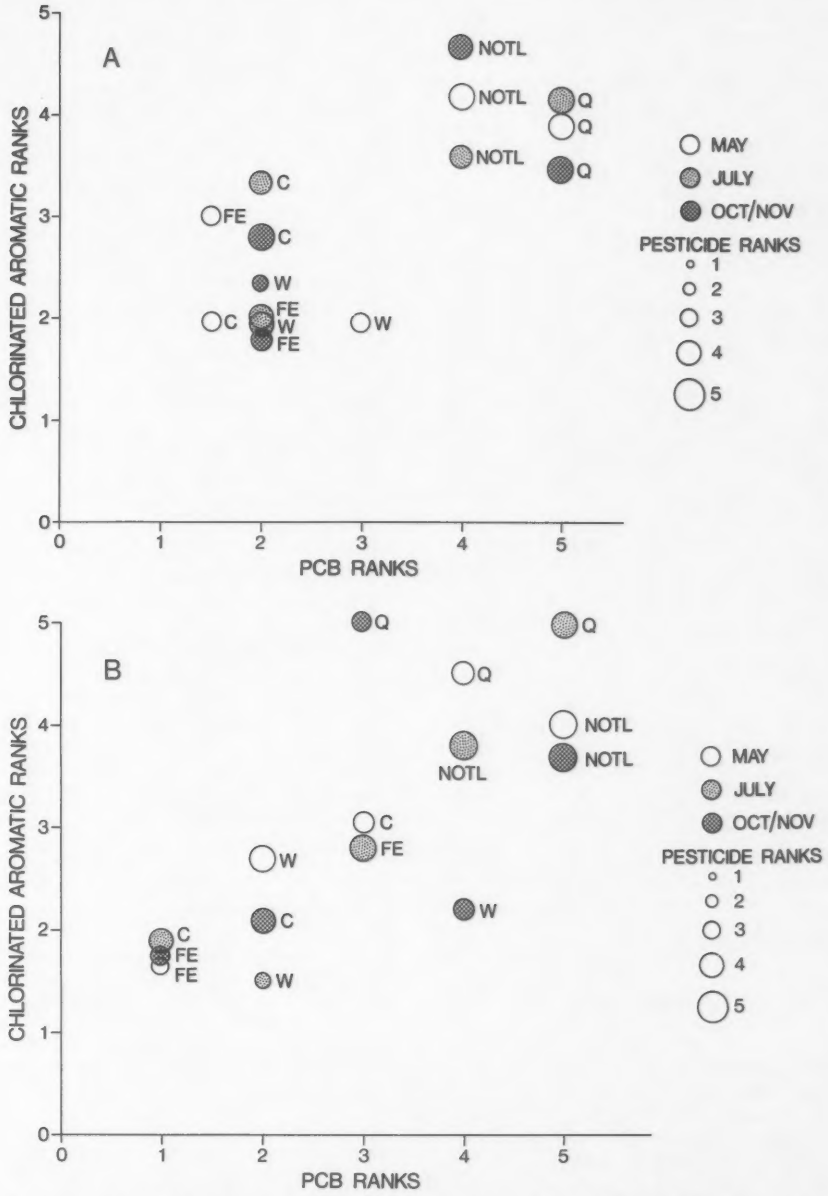

FIG. 5. Ranks for chlorinated aromatics, pesticides, and PCBs by sampling location and date for (A) WQB suspended sediment data and (B) WQB aqueous phase data.

Separate comments are appropriate for the individual data sets. The least powerful statistical method was used for the drinking water data, since this was the least quantitative of the three sets. Further, the samples were collected over a number of years, across seasons within a year, and over a long enough time to make changes in analytical methodology of concern. The sign test, as used here, is a simple procedure that can accommodate these limitations in the data. By comparing the level of a substance at NOTL and FE on matched sampling dates, the above-mentioned effects due to sampling over time can be eliminated at least to the extent possible without further information. Since two nonquantitative responses, trace (Tr) and not detected (ND), were recorded as well as concentrations, ranking of the outcome from a pair of stations as described above is a procedure that retains the most information without introducing further assumptions. The more powerful signed-rank test could be used if we could rank pairs such as the following: (Tr, ND) and (0.25, 0.32). This may be feasible for certain sets of data but it was not further considered here.

In summary, the two most important features of the analysis of the drinking water data are (1) the use of the information about when the samples were collected to eliminate time effects

TABLE 6. Statistics for the differences between locations for individual substances (*significant at 5%; **significant at 1%; ***significant at 0.1%; BD = binomially distributed).

Parameter	Suspended phase S_1	Aqueous phase S_1	Combined S_1
Total PCBs	157**	159**	316**
Pesticides			
Aldrin	BD	BD	BD
Dieldrin	131	136	267
A-BHC	154.5*	149	303.5**
G-BHC (Lindane)	BD	155**	—
A-Chlordane	133	136	269
G-Chlordane	BD	BD	BD
o,p-DDT	BD	BD	BD
p,p-DDT	BD	BD	BD
p,p-DDE	131	152*	284
p,p-DDD (TDE)	BD	BD	130.5
I-Endosulfan	145	141	286
II-Endosulfan	129	BD	—
Endrin	132.5	135	267.5
Heptachlor	BD	BD	BD
Heptachlor Epoxide	153*	95	248*
Methoxychlor	142	—	—
Mirex	157**	87	244*
Chlorinated aromatics			
1,2-Dichlorobenzene	152.5*	137	289.5
1,3)Dichlorobenzene	133	144	277
1,4-Dichlorobenzene	95	151*	246*
1,2,3-Trichlorobenzene	158**	159**	317***
1,2,4-Trichlorobenzene	155**	154*	309***
1,2,5-Trichlorobenzene	129.5	152*	281.5
1,2,3,4-Tetrachlorobenzene	157**	154*	311***
1,2,3,5- and 1,2,4,5-Tetrachlorobenzene	159**	155**	314***
Pentrachlorobenzene	157**	157**	314.5***
Hexachlorobenzene	156**	154*	310***
Metals			
Mercury	161**	—	—
Arsenic	143	—	—
Selenium	BD	—	—

and (2) the ranking of the outcome of a pair as a means of using the sign test when differences between quantitative levels cannot be calculated for all pairs. Application of the sign test, in a similar fashion, would often permit tests of significance to be performed where this is not presently being done. Since no assumptions about the distribution of the concentration of a substance need be made, if applied in keeping with the design of the data collection, the sign test should increase confidence in our conclusions.

Although the question of primary concern here is a difference in level between two regions of the Niagara River, a more extensive analysis might include the examination of the constancy of the difference over time. The plot of the cumulative sum for the number of pairs in which the NOTL level was above that of FE is the type of plot that can also be applied to ranks or quantitative data.

In contrast with the drinking water data, where useable records for individual substances were of varying length, sometimes providing many measurements for one substance, data were obtained at only three times in the WQB study, but results were reported for all substances each time. Thus, univariate procedures, used exclusively with the drinking water data, are supplemented by multivariate procedures that compare the results at different stations for all the substances simultaneously. Since the outcome was either ND or a concentration, more powerful rank procedures could be applied. For each substance, at any one sampling time, the five locations were ranked according to the level of the substance. The clustering of locations based on the Euclidean distance identified stations with the most similar ranks over all substances. The tests of the hypothesis of no difference in individual substances due to location were possible, since five locations were sampled even though only three sampling times were used. The use of these two types of methods together shows clearly which stations are different and for which substances.

Parametric procedures are preferable when distributional assumptions can be satisfied, and thus, normal-theory methods were used with the four tested metals. The size of the difference in concentration that can be measured is so small relative to the level of concentration for the other four metals that one would not expect to be able to detect differences between stations. If differences between means for each day are examined, month effects can be eliminated. Using the sign test with these differences, the significance levels for the one-sided test of the hypothesis of no difference are 0.94, 0.34, 0.81, and 0.88 for nickel, chromium, cadmium, and lead, respectively. Application of the more powerful Wilcoxon signed-rank test (Lehmann and D'Abrera 1975) is neither warranted, in view of the results of the sign test, nor practicable, due to ties, for these metals.

The total strength of the evidence of differences between the upper and lower Niagara River must be based on a synthesis of the results from individual data sets. A measure of uncertainty can be attached to the conclusions from the individual data sets but not to the conclusion based on all the data sets. This is not peculiar to the Niagara River data sets.

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References

ALLAN, R. J., A. MUDROCH, AND M. MUNAWAR [ED.] 1983. The Niagara River – Lake Ontario pollution problem. J. Great Lakes Res. 9: 232 p.

ANDERBERG, M. R. 1973. Cluster analysis for applications. Academic Press, New York and London. 359 p.

BENNETT, C. A., AND N. L. FRANKLIN. 1954. Statistical analysis in chemistry and the chemical industry. John Wiley & Sons, Inc., New York, London, Sydney. 724 p.

BROWNLEE, K. A. 1965. Statistical theory and methodology in science and engineering. John Wiley & Sons, Inc., New York, London, Sydney. 590 p.

DATA QUALITY SUBCOMMITTEE. 1984. Final report of DQS to Niagara River Toxics Committee. Inland Waters Directorate, Ontario Region, Water Quality Branch, Burlington, Ont.

DRAPER, N. R., AND H. SMITH. 1981. Applied regression analysis. John Wiley & Sons, Inc., New York, Chichester, Brisbane, Toronto, Singapore. 709 p.

ENVIRONMENT CANADA. 1979. Analytical methods manual. Water Quality Branch, Inland Waters Directorate, Ottawa, Ont.

HOLLANDER, M., AND D. A. WOLFE. 1973. Nonparametric statistical methods. John Wiley & Sons, Inc., New York, London, Sydney, Toronto. 503 p.

KENDALL, M. G., AND A. STUART. 1968. The advanced theory of statistics. Vol. 3. Design and analysis, and time series. Hafner Publishing Company, New York, NY. 557 p.

KUNTZ, K. W., AND N. D. WARRY. 1983. Chlorinated organic contaminants in water and suspended sediments of the Lower Niagara River. J. Great Lakes Res. 9(2): 241–248.

LEHMANN, E. L., AND H. J. M. D'ABRERA. 1975. Nonparametrics, statistical methods based on ranks. Holden Day Inc., San Francisco, CA. 457 p.

MCCREA, R. C. 1982. Development of an aqueous phase liquid – liquid extractor (APLE). Interim Report. Inland Waters Directorate, Ontario Region, Water Quality Branch, Burlington, Ont.

ONTARIO MINISTRY OF THE ENVIRONMENT (MOE). 1984. Analytical methods outline handbook. Laboratory Service Board, Toronto, Ont.

PUTTER, J. 1955. The treatment of ties in some nonparametric tests. Ann. Math. Stat. 26: 368–386.

SCHEFFÉ, H. 1959. The analysis of variance. John Wiley & Sons, Inc., Chichester, Brisbane, Toronto, Singapore. 477 p.

Adjuvant-Dependent Immunity and the Agglutinin Response of Fishes Against *Aeromonas salmonicida*, Cause of Furunculosis

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Adjuvant-dependent immunity resulted in agglutinin activity against *Aeromonas salmonicida* in sera of convalescent fishes. Rainbow trout (*Salmo gairdneri*), brown trout (*Salmo salar*), and brook trout (*Salvelinus fontinalis*) that had recovered from natural furunculosis produced serum agglutinin antibodies predominately against a specific chromatographic fraction (EcF1) of the bacterium's extracellular product. EcF1 was resolved by ion-exchange chromatography from DEAE Sephadex A-25. When EcF1 was emulsified in Freund's incomplete adjuvant and injected intraperitoneally, brook trout were protected against experimental challenge; brook trout vaccinated with EcF1 only were not protected against furunculosis. EcF1, examined by immunodiffusion tests, SDS – polyacrylamide gel electrophoresis, and Western blot immunoassays, shared certain properties with bacterial endotoxin. EcF1 and endotoxin preparations both contained immunoreactive lipopolysaccharides which indicated that adjuvant-dependent immunity to *A. salmonicida* is related to endotoxin.

L'immunité liée à un adjuvant a entraîné l'activation de l'agglutinine contre *Aeromonas salmonicida* dans le sérum de poissons convalescents. Des truites arc-en-ciel (*Salmo gairdneri*), des saumons atlantiques (*Salmo salar*) et des ombles de fontaine (*Salvelinus fontinalis*) qui s'étaient rétablis de furonculose naturelle ont produit des anticorps à l'agglutinine sérique, surtout contre une fraction chromatographique particulière (EcF1) du produit bactérien extracellulaire. Cette fraction a été obtenue de DEAE Sephadex A-25 par chromatographie sur échangeurs d'ions. Avec une injection intrapéritonéale de EcF1, émulsifié dans l'adjuvant incomplet de Freund, les ombles de fontaine étaient protégées contre la furonculose; celles qui avaient été vaccinées avec EcF1 seulement ne l'étaient pas. Soumis à des tests d'immunodiffusion, à l'électrophorèse au gel de polyacrylamide – SDS et à des épreuves immunologiques, le EcF1 a révélé certaines propriétés communes avec l'endotoxine bactérienne. Les préparations de EcF1 et d'endotoxine contenaient des lipopolysaccharides immunoréactifs, ce qui porte à croire que l'immunité liée à un adjuvant contre *A. salmonicida* est reliée à l'endotoxine.

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Aeromonas salmonicida is a gram-negative nonmotile bacterium that infects and initiates diverse pathological conditions in different families of fishes. The bacterium typically induces furunculosis — a systemic infection that limits the intensive culture of salmonid fishes. Many strains of *A. salmonicida* are resistant to chemotherapeutants (e.g. sulfamerazine and oxytetracycline) that are registered for treatment of infected food fish (Aoki et al. 1971; Snieszko and Bullock 1957). Therefore, chemotherapy is commonly ineffective.

Vaccination programs have been initiated to prevent furunculosis, but unless the vaccine is administered by injection (Krantz et al. 1964), equivocal results are obtained (Paterson 1981). Production of furunculosis vaccines is complicated by the lack of practical delivery systems. Recently, Cipriano and Starliper (1982) successfully vaccinated both brook trout (*Salvelinus fontinalis*) and Atlantic salmon (*Salmo salar*) against furunculosis by immersion in an attenuated strain of *A. salmonicida*. Cipriano (1982a) also indicated that a cytolytic glycoprotein associated with soluble extracts of *A. salmonicida* is immunogenic in brook trout. However, little information exists on the protective nature of other antigens from *A. salmonicida* for fish. *Aeromonas salmonicida* has endotoxin (Paterson and Fryer 1974a), proteases (Sakai 1977; Shieh and MacLean 1975), leukocytolytic factor (Fuller et al. 1977), hemolysin (Nomura and Saito 1982), and an additional protein layer associated with its outer membrane (Kay et al. 1981; Udey and Fryer 1978). Although some of these components are antigenic, the importance of these antigens for vaccination is not clearly understood.

Several immunologic activities are stimulated by vaccination. However, the agglutinin activity, and only that activity, is associated with specific immunoglobulin IgM-like antibodies (Cisar and Fryer 1974; Dorson 1972; Ikeda 1978). Protective

agglutinins were developed in coho salmon (*Oncorhynchus kisutch*) that had been injected intraperitoneally (i.p.) with formalin-inactivated *A. salmonicida* mixed with Freund's complete adjuvant (Paterson and Fryer 1974b). Similar preparations of purified endotoxin injected into coho salmon produced specific antibodies, but the protective nature of the antibodies was unclear. Michel (1979) later noted that the presence of humoral antibodies in vaccinated fish was not necessarily indicative of protection. However, humoral antibodies against *A. salmonicida* can be passively transferred to normal fish and therein provide protection against experimental furunculosis (Cipriano 1982b; Spence et al. 1965).

The purpose of our study was to further examine details of the agglutinin response of trout against specific antigens from *A. salmonicida*. Convalescent rainbow trout (*Salmo gairdneri*), brown trout (*Salmo trutta*), and brook trout were used to identify antigens that induced antibody activity. The protective nature of the candidate antigens was further examined by immunization trials with brook trout.

Materials and Methods

Aeromonas salmonicida, strain 3.101, isolated from diseased fish at the National Fish Hatchery (Kearneysville, WV) in September 1978, and maintained in the culture collection at the National Fish Health Research Laboratory (Kearneysville, WV) was used throughout this study. Washed and unwashed cells used as antigens in immunoassays were produced as described by Cipriano (1982a).

In addition, cell-free extracts (CFE) were precipitated from spent media by the addition of dry $(NH_4)_2SO_4$ crystals to give 80% saturation, as described elsewhere (Cipriano et al. 1981). The precipitate was sedimented by centrifugation at 3896 × *g* for 30 min at 4°C, the supernatant was decanted, and the precipitate was redissolved in 10.0 mL of triple-distilled water. This suspension was dialyzed against sterile 0.01 mol K_2HPO_4/L (pH 8.0) for 18 h at 4°C, and diluted to 20 mL with triple-distilled water. This suspension, estimated to contain 1.4 mg protein/mL (Bradford 1976), was used as a crude CFE antigen from *A. salmonicida*, was stored at 4°C, and used at a concentration of 50 μg protein/mL.

Part of the CFE antigen (10 mL) was fractionated by ion-exchange chromatography (Cipriano et al. 1981). Four fractions were thus eluted from DEAE Sephadex A-25 columns using a discontinuous NaCl gradient. Each of the four fractions, designated EcF1, 2, 3, 4, was adjusted in phosphate-buffered saline (PBS) to contain 50 μg protein/mL (Bradford 1976). Protein profiles were consistent with previous reports (Cipriano 1982a; Cipriano et al. 1981).

To determine which antigen best evoked an agglutinin response in fish, the sera from rainbow, brook, and brown trout (94 g) were used. The fish were obtained from a hatchery where furunculosis is enzootic. Sera were pooled from five fish, and three such pools were taken from each species. Sera were assayed for agglutinin activity against the antigens. Agglutinin activity was determined initially by standard microtiter agglutination against either the unwashed or washed whole cell antigen. Additionally, each of the four CFE fractions was adsorbed onto sheep erythrocytes. The sera were also assayed for passive hemagglutination activity using the method of Sundick and Rose (1980).

Next, EcF1 was emulsified 1:1 (v/v) in Freund's incomplete adjuvant. The immunogenic nature of this antigen was assessed in specific pathogen-free brook trout (54 g). Each of 16 brook trout in three groups was injected i.p. with 0.2 mL of the EcF1 adjuvant preparation (5 μg EcF1/fish). Each of 16 additional brook trout were similarly injected with 5 μg EcF1 in 0.2 mL of PBS, and still other brook trout were injected i.p. with 0.2 mL of a 1:1 (v/v) mixture of PBS and Freund's incomplete adjuvant. Immunization experiments were duplicated, and after 21 d all fish were challenged by a 60-s exposure to 1.3×10^9 virulent *A. salmonicida*/mL, as described by Cipriano (1982b). Mortality was recorded for 14 d after challenge, and the presence of *A. salmonicida* was confirmed by examining kidney smears of dead fish by direct fluorescent antibody technique (Bullock et al. 1980).

To determine if EcF1 was antigenically similar to this bacterium's endotoxin, equal concentrations of EcF1 and purified endotoxin, prepared by the method of Westphal and Jann (1965), were placed in adjacent wells on immunodiffusion plates and reacted with a rabbit antiserum to whole cells of *A. salmonicida*. Plates were allowed to react for 18 h at 4°C and then examined for precipitin bands. The presence of endotoxin in both preparations was detected by the *Limulus* amebocyte lysate assay (E-Toxate, Sigma Chemical Co., St. Louis, MO). EcF1 and endotoxin were further characterized by polyacrylamide gel electrophoresis. Polyacrylamide slab gels (1.5 mm thick) were cast as described by Laemmli (1970) using a 10% acrylamide resolving gel and 4% acrylamide in the stacking gel. Samples of EcF1, endotoxin, and the appropriate molecular weight markers were boiled for 5 min in sample buffer, and 10 μL of each sample were loaded into gel slots. Electrophoresis was begun at 15 mA until the bromphenol blue tracking dye reached the interface with the resolving gel. The current was then increased to 25 mA with a 200-V limit. After electrophoresis at room temperature, gels were stained in 0.01% Coomassie blue in 25% methanol, 10% acetic acid, and destained in the same solution without the dye. Silver stain for lipopolysaccharides (LPS) was by the method of Tsai and Frasch (1982).

Polyacrylamide gels used in protein blotting experiments were transferred immediately to 200 mL of electrode buffer, as previously described for the electrotransfer of proteins from SDS–gels by Towbin et al. (1979).

Antigens transferred to the nitrocellulose (NC) sheets were visualized by the Coomassie blue stain procedure, described previously, or by reactions with enzyme-labelled antisera. For immunological detection of antigens, the NC sheets were soaked for 10 min in 20 mmol Tris/L, 500 mmol NaCl/L, pH 7.5 (Tris-buffered saline (TBS)). Unreacted sites on the NC were blocked by soaking the NC sheets in 3% gelatin in TBS for 30 min. Pooled rabbit IgG against whole cells of *A. salmonicida* was diluted 1:50 in 1% gelatin in TBS and incubated, with gentle agitation, with the NC sheets for 16 h at room temperature. After a brief rinse in distilled water, NC sheets were washed in two changes of TBS for 20 min. The NC was then incubated for 1 h in a 1:3000 dilution of goat anti-rabbit IgG horseradish peroxidase conjugated in 1% gelatin–TBS at room temperature. NC sheets were washed in distilled water and TBS, as previously described, and developed with 0.015% H_2O_2 and 4-chloro-1-naphthol for 5–10 min. Reagents and protocols for the immunodetection procedure were obtained from Bio-Rad Laboratories.

Results and Discussion

Our initial results indicated that the sera from convalescent fishes had far greater agglutinin titers against unwashed cells of *A. salmonicida* than against the washed cell antigen (Table 1).

TABLE 1. Agglutinin titers among pooled samples of serum[a] from three species of fishes that survived natural epizootics of furunculosis against cellular antigens of *A. salmonicida*.

Antigen	Log (2) dilution titer[b] of pooled serum		
	Rainbow trout	Brown trout	Brook trout
Washed cells	3	0	3
	4	4	4
	4	5	4
Unwashed cells	9	8	9
	10	10	10
	9	9	9

[a]Five fish per serum pool.
[b]Reciprocal of last dilution with positive agglutination.

TABLE 2. Passive hemagglutinin titers among pool samples of serum[a] from three species of fishes that survived natural epizootics of furunculosis against individual chromatographic fractions (EcF1–4) of a cell-free extract of *A. salmonicida*.

Species	Log (2) hemagglutinin dilution titers[b] against fractions			
	1	2	3	4
Rainbow trout	8	2	2	1
	10	1	1	0
	9	2	2	1
Brown trout	8	1	1	1
	9	1	3	0
	9	2	3	1
Brook trout	9	1	1	1
	10	1	2	1
	11	1	2	1

[a]Five fish per serum pool.
[b]Reciprocal of last dilution with positive hemagglutination.

The geometric mean of serum agglutinin titers against the washed antigens was 3.6 for rainbow trout, 3 for brown trout, and 3.6 for brook trout. In contrast, each species of trout had considerably higher antibody titers against unwashed whole cell antigen. The mean of serum titers from rainbow trout and brook trout was 9.3 and from brown trout was 9.0.

Because fractions derived from the CFE of *A. salmonicida* were soluble in nature, the antibody response to these components was determined by passive hemagglutination. These assays indicated that agglutination response was directed primarily against EcF1 (Table 2). The mean of hemagglutinin titers against EcF1 were 9 among rainbow trout, 8.7 among brown trout, and 10 among brook trout. However, in each of the three species, serum agglutinin titers ranged only from 0.7 to 2.3 against fractions 2, 3, and 4.

Results obtained with EcF1 in the immunization trials were comparable with those obtained by others using whole cell vaccines (Michel 1979; Paterson and Fryer 1974b). Krantz et al. (1964) showed that both rabbits and brook trout produced appreciably higher serum agglutinin titers when injected with cells of *A. salmonicida* mixed in adjuvant than experimental animals injected only with bacteria. Additionally, these researchers noted that brook trout vaccinated with formalin-killed cells of

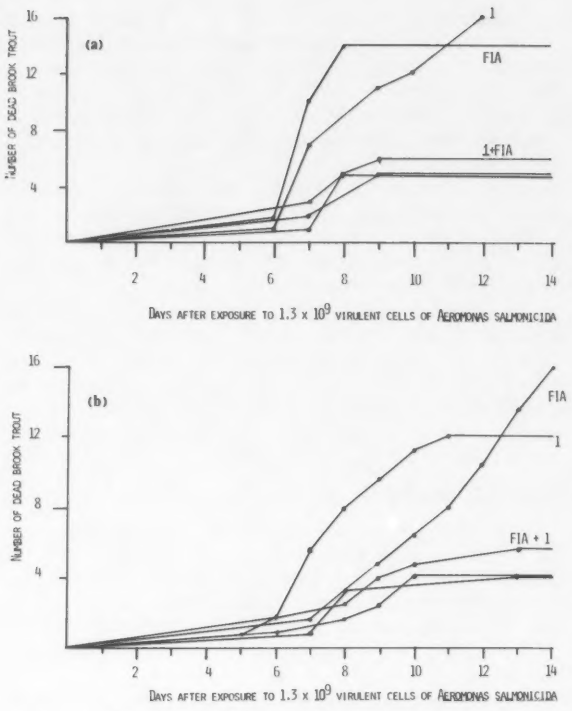

FIG. 1. (a) Mortality among groups of 16 brook trout vaccinated with EcF1 derived from a cell-free extract of *A. salmonicida* (1), EcF1 mixed with Freund's incomplete adjuvant (1 + FIA), or injected with only the adjuvant (FIA). (b) Replicate of the experiment illustrated in Fig. 1a indicating the repeatability of protection by EcF1 emulsified in adjuvant.

A. salmonicida administered with adjuvant became immune to furunculosis, whereas if adjuvant was omitted the fish were susceptible to the disease. Our results suggest that an antigen contained in EcF1 may account for the adjuvant-related protection observed with whole cell vaccine.

Cipriano (1982a) was unable to show that EcF1 injected without adjuvant was immunogenic. Therefore, the ability of EcF1 to produce immunity when injected with adjuvant was assessed. These studies indicated that brook trout immunized with EcF1 mixed with adjuvant were protected against experimental challenge, but trout injected only with EcF1 were not protected (Fig. 1a). In the three groups of fish vaccinated with EcF1 plus Freund's incomplete adjuvant, statistically significant protection was established ($\chi^2_{(1\,df)} \geq 10.8$; $P < 0.01$) whereas fish vaccinated with EcF1 alone were not protected against experimental challenge ($\chi^2_{(1\,df)} = 0.53$; $P > 0.05$) as compared with the mortality among control trout injected with Freund's incomplete adjuvant.

A duplicate experiment was done to confirm the reproducibility of these results (Fig. 1b). Protection was not significant ($P > 0.05$) among brook trout injected only with EcF1 ($\chi^2_{(1\,df)}$ 5.9) but highly significant among the three groups of brook trout injected with EcF1 plus adjuvant ($\chi^2_{(1\,df)} \geq 17.6$; $P < 0.01$).

Rabbit antiserum prepared against unwashed cells of *A. salmonicida* formed two precipitin bands against EcF1, one of which continued to form a band of partial identity with endotoxin (Fig. 2). Because EcF4 of the CFE from *A. salmonicida*

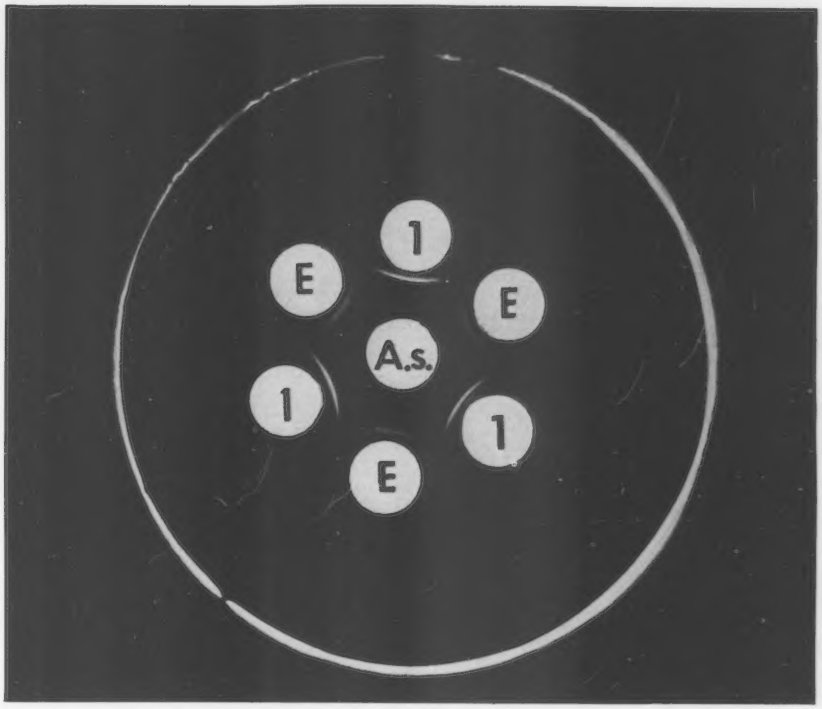

FIG. 2. Immunodiffusion of EcF1 (1) derived from a cell-free extract of *A. salmonicida* and preparations of bacterial endotoxin (E) against a rabbit antiserum to unwashed cells of *A. salmonicida* (A.s.).

was previously shown to be immunogenic in brook trout, a specific rabbit antiserum to EcF4 (Cipriano 1982a) was also reacted on an immunodiffusion plate with EcF1 and endotoxin. This specific antiserum, however, did not form precipitin bands with endotoxin or EcF1. Whereas endotoxin and EcF1 shared a common antigen, the component was different from EcF4 (Cipriano 1982a).

The presence of LPS in both EcF1 and the endotoxin was demonstrated by the *Limulus* lysate assay. Both samples congealed within 4 h at 37°C, whereas control tubes were negative.

Electrophoresis demonstrated that endotoxin and EcF1 are similar mixtures of proteins. However, the resolution of both preparations was consistently poor in the range of molecular weight above 50 000 through multiple experiments with varying loading volumes. This lack of resolution evidenced by protein stains indicated that nonprotein material was present in the higher molecular weight components.

Replicate gels, electroblotted onto NC membranes and reacted with a rabbit antiserum against whole unwashed cells of *A. salmonicida*, indicated that immune serum reacted with an antigen in EcF1 that correlated with the LPS bands of silver-stained electrophorograms (Fig. 3, region A). Region A of the endotoxin preparation was similarly antigenic, but additional antigens were also detected in the endotoxin that were not visualized in EcF1 (Fig. 3, region B). Although both samples were roughly equilibrated by protein content, both silver and immunologic staining intensities apparently reflect quantitative differences in LPS concentrations.

Results obtained with EcF1 in the immunization trials were comparable with those obtained by others using whole cell vaccines (Michel 1979; Paterson and Fryer 1974b).

Krantz et al. (1964) showed that both rabbits and brook trout produced appreciably higher serum agglutinin titers when injected with cells of *A. salmonicida* mixed in adjuvant than experimental animals injected only with bacterial cells. Additionally, these researchers noted that brook trout vaccinated with formalin-killed cells of *A. salmonicida* administered with adjuvant became immune to furunculosis, whereas if adjuvant was omitted, the fish were susceptible to the disease. Our results suggest that an antigen contained in EcF1 may account for the adjuvant-related protection observed with whole cell vaccines.

Results of our immunodiffusion tests, polyacrylamide gel electrophoresis tests, and immunoblot assays noted that certain similarities existed between EcF1 and endotoxin. The presence of endotoxin in EcF1 of the CFE from *A. salmonicida* was not alarming. Paterson (1972) found that some endotoxin from *A. salmonicida* was sloughed into culture media as a result of cellular autolysis during stationary and death phases of culture. In fact, spent cell-free culture media have been used as a source of free endotoxin (Paterson and Fryer 1974a). Because our CFE antigen was harvested from cultures of *A. salmonicida* during its stationary phase, some endotoxin could have been present in the crude extract. Although similarities exist between EcF1 and endotoxin, both preparations contain several components. Because of this complexity, we identified that the immunoreactive areas of EcF1 were identical to endotoxin. Additively, *Limulus* lysate and silver stain assays detected LPS in EcF1. Because we were able to visualize only LPS and not protein antigens by immunodetection after electrotransfer onto NC, we concluded

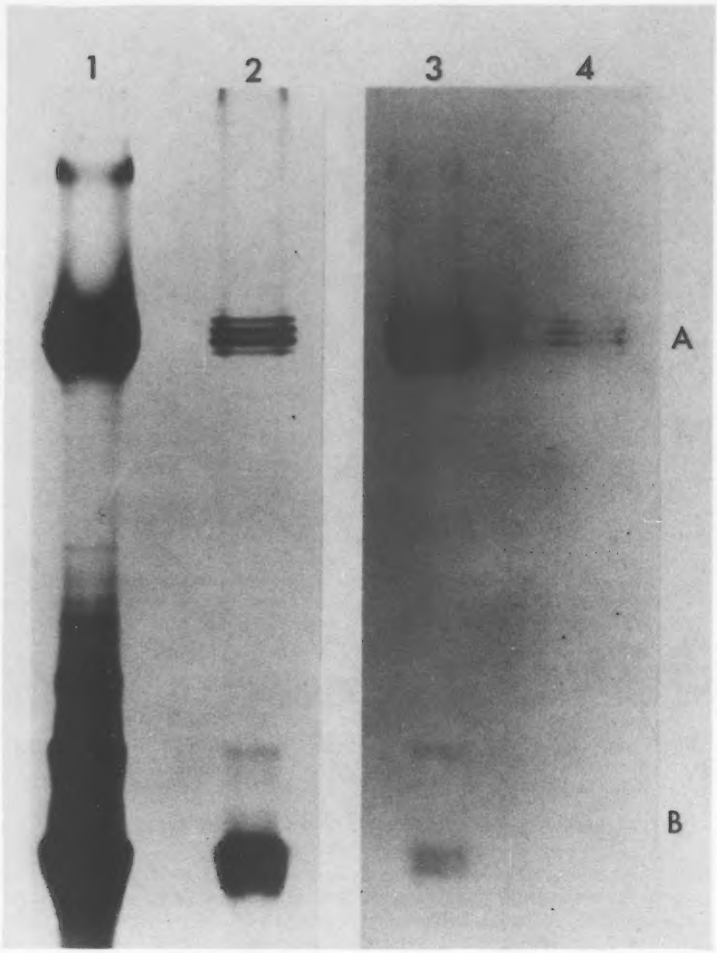

FIG. 3. SDS – polyacrylamide gel electrophoresis of *A. salmonicida* preparations comparing silver-stained gel with specific immunoperoxidase-stained nitrocellulose (NC) replica. Lane 1, *A. salmonicida* endotoxin stained with silver nitrate, 0.2 ng; lane 2, *A. salmonicida* EcF1 from DEAE A-25 chromatography stained with silver nitrate, 0.3 ng; lane 3, same as lane 1 electroblotted to NC and reacted with rabbit antiserum to *A. salmonicida*; lane 4, same as lane 2 electroblotted to NC and reacted with rabbit antiserum to *A. salmonicida*. Gel concentration was 10% total acrylamide. Sample concentration was determined by Coomassie blue binding relative to normal human serum standards.

that the protective nature of EcF1 in trout is directly related to adjuvant-dependent immunity to endotoxin.

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References

AOKI, T., S. EGUSA, T. KIMURA, AND T. WATANABE. 1971. Detection of R factors in naturally occurring *Aeromonas salmonicida* strains. Appl. Microbiol. 22: 716–717.

BRADFORD, M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein–dye binding. Anal. Biochem. 72: 248–254.

BULLOCK, G. L., B. R. GRIFFIN, AND H. M. STUCKEY. 1980. Detection of *Corynebacterium salmoninus* by direct fluorescent antibody test. J. Fish. Res. Board Can. 37: 719–721.

CIPRIANO, R. C. 1982a. Immunogenic potential of growth products from cultures of *Aeromonas salmonicida* for brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 39: 1512–1518.

1982b. Immunization of brook trout (*Salvelinus fontinalis*) against *Aeromonas salmonicida*: immunogenicity of virulent and avirulent isolates and protective ability of different antigens. Can. J. Fish. Aquat. Sci. 39: 218–221.

CIPRIANO, R. C., B. R. GRIFFIN, AND B. C. LIDGERDING. 1981. *Aeromonas salmonicida*: relationship between extracellular growth products and isolate virulence. Can. J. Fish. Aquat. Sci. 38: 1322–1326.

CIPRIANO, R. C., AND C. E. STARLIPER. 1982. Immersion and injection vaccination of salmonids against furunculosis with an avirulent strain of *Aeromonas salmonicida*. Prog. Fish-Cult. 44: 167–169.

CISAR, J. O., AND J. L. FRYER. 1974. Characterization of anti-*Aeromonas salmonicida* antibodies from coho salmon. Infect. Immun. 9: 236–243.

DORSON, M. 1972. The immune response of rainbow trout: several characteristics of immunoglobulin production during the primary response. Ann. Rech. Vet. 3: 93–107.

FULLER, D. W., K. S. PILCHER, AND J. L. FRYER. 1977. A leukocytolytic factor isolated from cultures of *Aeromonas salmonicida*. J. Fish. Res. Board Can. 34: 1118–1125.

IKEDA, K. 1978. Gel chromatographic characteristics of agglutinating antibody of rainbow trout to *Aeromonas salmonicida*. Bull. Freshwater Fish. Res. Lab. (Tokyo) 28: 55–60.

KAY, W. W., J. T. BULKLEY, E. E. ISHIGURO, B. M. PHIPPS, J. D. L. MONETTE, AND T. J. TRUST. 1981. Purification and disposition of a surface protein associated with virulence of *Aeromonas salmonicida*. J. Bacteriol. 147: 1077–1084.

KRANTZ, G. E., J. M. REDDECLIFFE, AND C. E. HEIST. 1964. Immune response of trout to *Aeromonas salmonicida*. Part I. Development of agglutinating antibodies and protective immunity. Prog. Fish-Cult. 26: 3–10.

LAEMMLI, U. K. 1970. Cleavage of structural protein during the assembly of the head of bacteriophage T4. Nature (Lond.) 227: 680–685.

MICHEL, C. 1979. Furunculosis of salmonids: vaccination attempts in rainbow trout (*Salmo gairdneri*) by formalin-killed germs. Ann. Rech. Vet. 10: 33–40.

NOMURA, S., AND H. SAITO. 1982. Production of the extracellular hemolytic toxin by an isolated strain of *Aeromonas salmonicida*. Bull. Jpn. Soc. Sci. Fish. 48: 1589–1597.

PATERSON, W. D. 1972. The antibody response of juvenile coho salmon (*Oncorhynchus kisutch*) to *Aeromonas salmonicida*, the causative agent of furunculosis. Ph.D. thesis, Oregon State University, Corvallis, OR. 100 p.

1981. *Aeromonas salmonicida* as an immunogen, p. 375–385. *In* D. P. Anderson and W. Hennessen [ed.] Proceedings of the International Symposium on Fish Biologics: Serodiagnostics and Vaccines, National Fish Health Research Laboratory, Kearneysville, WV, 26–30 April 1980.

PATERSON, W. D., AND J. L. FRYER. 1974a. Effect of temperature and antigen dose on the antibody response of juvenile coho salmon (*Oncorhynchus kisutch*) to *Aeromonas salmonicida* endotoxin. J. Fish. Res. Board Can. 31: 1743–1749.

1974b. Immune response of juvenile coho salmon (*Oncorhynchus kisutch*) to *Aeromonas salmonicida* cells administered intraperitoneally in Freund's complete adjuvant. J. Fish. Res. Board Can. 21: 1751–1755.

SAKAI, D. K. 1977. Causative factor of *Aeromonas salmonicida* in salmonid furunculosis: extracellular protease. Sci. Rep. Hokkaido Fish Hatchery 32: 61–89.

SHIEH, H. S., AND J. R. MACLEAN. 1975. Purification and properties of an extracellular protease of *Aeromonas salmonicida*, the causative agent of furunculosis. Int. J. Biochem. 6: 653–656.

SNIESZKO, S. F., AND G. L. BULLOCK. 1957. Treatment of sulfonamide resistant furunculosis in trout and determination of drug sensitivity. U.S. Fish Wildl. Fish. Bull. 57: 555–564.

SPENCE, K. D., J. L. FRYER, AND K. S. PILCHER. 1965. Active and passive immunization of certain salmonidc fishes against *Aeromonas salmonicida*. Can. J. Microbiol. 11: 397–405.

SUNDRICK, R. S., AND N. R. ROSE. 1980. Agglutination reactions, p. 109–127. *In* N. R. Rose and P. E. Bigazzi [ed.] Methods in immunodiagnosis. John Wiley & Sons, New York, NY.

TOWBIN, H., T. STAEHELIN, AND J. GORDON. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. U.S.A. 76: 4350–4354.

TSAI, C. M., AND C. E. FRASCH. 1982. A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels. Anal. Biochem. 119: 115–119.

UDEY, L. R., AND J. L. FRYER. 1978. Immunization of fish with bacterins of *Aeromonas salmonicida*. Mar. Fish. Rev. 40: 12–17.

WESTPHAL, O., AND K. JANN. 1965. Bacterial lipopolysaccharides. Extraction with phenol–water and further applications of the procedure, p. 83–91. *In* R. L. Whistler [ed.] Carbohydrate chemistry. Vol. 5. General polysaccharides. Academic Press, New York and London.

Macroinvertebrate Abundance and Production of Psammophilous Chironomidae in Shifting Sand Areas of a Lowland River

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Soluk, D. A. 1985. Macroinvertebrate abundance and production of psammophilous Chironomidae in shifting sand areas of a lowland river. Can. J. Fish. Aquat. Sci. 42: 1296–1302.

Abundance and biomass of benthic macroinvertebrates from shifting sand areas in the bed of the Sand River in central Alberta, Canada, were examined for 1 yr. Macroinvertebrate density ranged from 12 000 to 78 000 individuals/m^2, but total biomass was low (50–490 mg/m^2 dry mass) due to the small size of most organisms. The interstitial larvae of two chironomid species (*Robackia demeijerei* and *Rheosmittia* sp.) contributed a mean of 80.6% biomass and 92.8% of total number of macroinvertebrates. Total annual secondary production of these two species (752.0 ± 144.5 $mg \cdot m^{-2} \cdot yr^{-1}$) was used as an estimate of total secondary production of benthic macroinvertebrates in shifting sand areas. Both *R. demeijerei* and *Rheosmittia* sp. exhibited larval growth and development rates much slower than those reported for comparably sized species in other habitats. Food or frequent disturbance may limit the growth of these species. Although unit area biomass and production were low relative to other lotic habitats, shifting sand areas make significant contributions to the river ecosystem because they occupy a large proportion of the river bed.

Le nombre et la biomasse de macroinvertébrés benthiques peuplant les sables mouvants du lit de la rivière Sand, au centre de l'Alberta (Canada), ont été étudiés pendant 1 an. La densité a varié de 12 000 à 78 000 individus/m^2, mais la biomasse totale était faible (de 50 à 490 mg/m^2 de masse sèche) à cause de la petite taille de la plupart des organismes. Les larves interstitielles de deux espèces de chironomidés (*Robackia demeijerei* et *Rheosmittia* sp.) représentaient en moyenne 80,6 % de la biomasse et 92,8 % du nombre total de macroinvertébrés. La production secondaire annuelle totale des deux espèces (752,0 ± 144,5 $mg \cdot m^{-2} \cdot an^{-1}$) a servi d'estimation de la production secondaire totale de macroinvertébrés benthiques dans les zones de sables mouvants. Chez *R. demeijerei* et *Rheosmittia* sp. les taux de croissance et de développement des larves étaient moins élevés que ceux qui étaient signalés chez des espèces de taille comparable fréquentant d'autres habitats. La croissance de ces espèces peut être entravée par le manque de nourriture ou les perturbations fréquentes. Quoique la biomasse et la production par unité de surface y soient faibles par rapport à d'autres habitats lotiques, les zones de sables mouvants fournissent une importante contribution à l'écosystème fluvial parce qu'elles recouvrent une grande partie du lit de la rivière.

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Attempts to study rivers as ecosystems require knowledge of functional relationships within and between communities of organisms occupying the various habitat types present in the river system. Most studies conducted in streams have concentrated on organisms and communities associated with relatively coarse substrates found in riffle areas. Such areas occupy only a small proportion of the total bed in large lowland rivers, systems usually dominated by fine-grained materials (sand and silt) and their characteristic bedforms (Leopold et al. 1964; Hynes 1970).

Shifting sand areas are one of the dominant types of substrate in the mainstream channel of most large lowland rivers. These areas are usually characterized by the presence of loosely consolidated sand that is formed into moving dunes by the force of the current, and thus present a dynamic substrate for colonization by benthic organisms. A well-developed microfauna has been reported from shifting sand areas (Niewestnova-Shadina 1935); however, these areas have been characterized as unfavourable habitats for benthic macroinvertebrates, supporting few individuals of a few specialized species (Hynes 1970). Studies of the effect of sand additions in smaller streams and rivers with predominantly stony bottoms (e.g. Nuttall 1972; Lenat et al. 1981) and many studies of unstable sand areas in large streams with predominantly sandy bottoms (Berner 1951; Zhadin and Gerd 1961; Sioli 1975; Northcote et al. 1976; Seagle et al. 1982) have supported this view. However, some recent studies (Barton and Lock 1979; Barton 1980; Benke et al. 1984), although supporting the view that shifting sands support few species, have reported high densities of small interstitial macroinvertebrates (mainly chironomid larvae).

Biomass values for shifting sand areas (Barton and Lock 1979; Benke et al. 1984) are low relative to most other lotic habitats, and this has often been interpreted as an indication of low productivity. However, Benke et al. (1984) estimated high rates of turnover for chironomid populations and thus obtained

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high values of secondary production for unstable sand areas in the Satilla River, Georgia.

My study assesses density and biomass of benthic macroinvertebrates on shifting sand areas in a lowland river (Sand River, Alberta), and evaluates the hypothesis of rapid turnover for populations of small chironomids in this habitat. The sum of estimates of production for the two dominant chironomid species is used as a minimal estimate of secondary production for the habitat. This value, in conjunction with data on trophic relations of the dominant macroinvertebrates in shifting sand areas of the Sand River, is used to examine the importance of shifting sand areas in river ecosystems.

Study Site

The study was carried out at the mouth of the Sand River (54°23′N, 111°02′W) in east-central Alberta, Canada. Immediately upstream of its mouth, the Sand River is approximately 30 m wide with mean thalweg current velocities ranging from 60 to 150 cm/s. Mean annual discharge over the study period was approximately 16 m^3/s. Much of the drainage basin is composed of diffuse sources (bogs, swamps, and eutrophic lakes), so its designation as a fifth-order stream, based on well-defined tributaries, is somewhat arbitrary.

Ice conditions persisted from early November until mid-April. Water temperature rose rapidly after breakup of ice, so that only 3 d after the last ice was apparent (April 23), temperature was 10°C. Mean daily water temperature from May 22 to September 26, 1981, was 19.5°C; maximum temperature recorded was 25°C.

The bottom of the Sand River is composed almost exclusively of sand, with silty areas near the banks and with gravel bars occurring infrequently in parts of the bed. More complete descriptions of the study site are given in Soluk (1983) and Soluk and Clifford (1984).

Methods

To determine macroinvertebrate density and biomass, I collected samples along 10 transects set out at 5-m intervals along a 50-m stretch of the river. Samples were obtained in shifting sand areas along these transects at intervals of 5, 10, 20, and 30 m from the east bank, wherever possible. A series of samples was obtained prior to freeze-up in 1980 (November 9) and approximately once every second week during the ice-free season of 1981 (April 23 to October 28).

The principal sampling device was a modified version of the core-freezer described by Shapiro (1958), with an internal diameter of 3.8 cm, and using a dry ice – butanol mixture as a freezing agent. A handled Surber sampler (mesh size 0.243 mm) was used exclusively on November 9, 1980. Cores were obtained by pushing the corer into the sediments to a depth of 25 cm. If cores were to be kept intact for sectioning, the water column and the core were separated; otherwise they were placed into the same container. All samples were frozen in the field and stored at −28°C until examination. Surber samples were preserved in 95% ethanol. Construction of the core-freezer and the techniques used to obtain Surber samples and cores are described in Soluk (1983).

To determine whether the corer collected all invertebrates in shifting sand areas, their vertical distribution in the sediment was examined. I sectioned cores that had been collected intact in September and October at 10, 15, and 20 cm from the top. Each of these sections was then treated as an individual sample and the number of macroinvertebrates in each section recorded.

TABLE 1. Mean head length and mean dry mass of all distinguishable larval instars of *R. demeijerei* and *Rheosmittia* sp.

Instar	Mean head length (μm)	No./sample	No. of samples	Mean dry wt (μg) ± SE
		Rheosmittia sp.		
I + II	55	20	45	0.7±0.3
III	74	10	53	1.7±0.8
IV	91	5	44	7.2±2.8
		R. demeijerei		
I	69	10	7	1.5±0.7
II	98	5	51	3.5±1.2
III	134	1	104	12.6±5.3
IV	194	1	57	29.1±13.2

Before processing, core samples were rapidly thawed in a warm dilute ethanol solution. For both types of samples, organic material was separated from inorganic material by elutriation (gold-panning technique) and concentrated on a 0.063-mm-mesh sieve. The organic material was then sorted under a dissecting microscope at 12× magnification and all benthic macroinvertebrates were removed and counted.

For determination of production and life history, larvae of the two dominant chironomid species, *Robackia demeijerei* (Kruseman) (Chironominae) and an undescribed species of *Rheosmittia* (D. R. Oliver, Biosystematics Res. Inst., Ottawa, Ont.) (Orthocladiinae), were separated by instar based on the head capsule length (measured from the posterior lateral margin to the base of the mandible), except for the first two instars of *Rheosmittia* sp., which were treated together since they were not separable on the basis of head capsule length.

Mean dry biomass for larval populations of *R. demeijerei* and *Rheosmittia* was calculated from counts and the mean dry mass for each instar (Table 1). All other macroinvertebrates were separated into two categories, "other chironomids" and "other organisms," and were weighed as a group to the nearest 0.002 mg. All organisms were dried at 60°C for 24 h before weighing.

Density estimates for the two dominant chironomids obtained with the Surber sampler on November 9, 1981, were corrected using efficiency estimates of 13 and 5% for *R. demeijerei* and *Rheosmittia* sp., respectively (Soluk 1983). Dry mass of total individuals in the sample was then estimated using these corrected values.

Larval production for *R. demeijerei* and *Rheosmittia* sp. was estimated using the size-frequency (Hynes) methods (Waters and Crawford 1973; Waters 1977). Production estimates obtained for *R. demeijerei* and *Rheosmittia* sp. larvae were multiplied by the number of generations per year to provide corrected estimates of annual production (Hamilton 1969). The 95% confidence intervals for each of the production estimates were determined using the method of Krueger and Martin (1980).

To determine diet, I qualitatively examined gut contents of *R. demeijerei* and *Rheosmittia* sp. larvae in slide-mounted specimens squashed to spread material in the guts. Uncleared specimens and specimens cleared in 10% potassium hydroxide were examined under a compound microscope.

Results

Mean total macroinvertebrate biomass from shifting sand areas of the Sand River (Fig. 1b) ranged between 50 and 492 mg/m^2, while mean density (Fig. 2b) ranged between

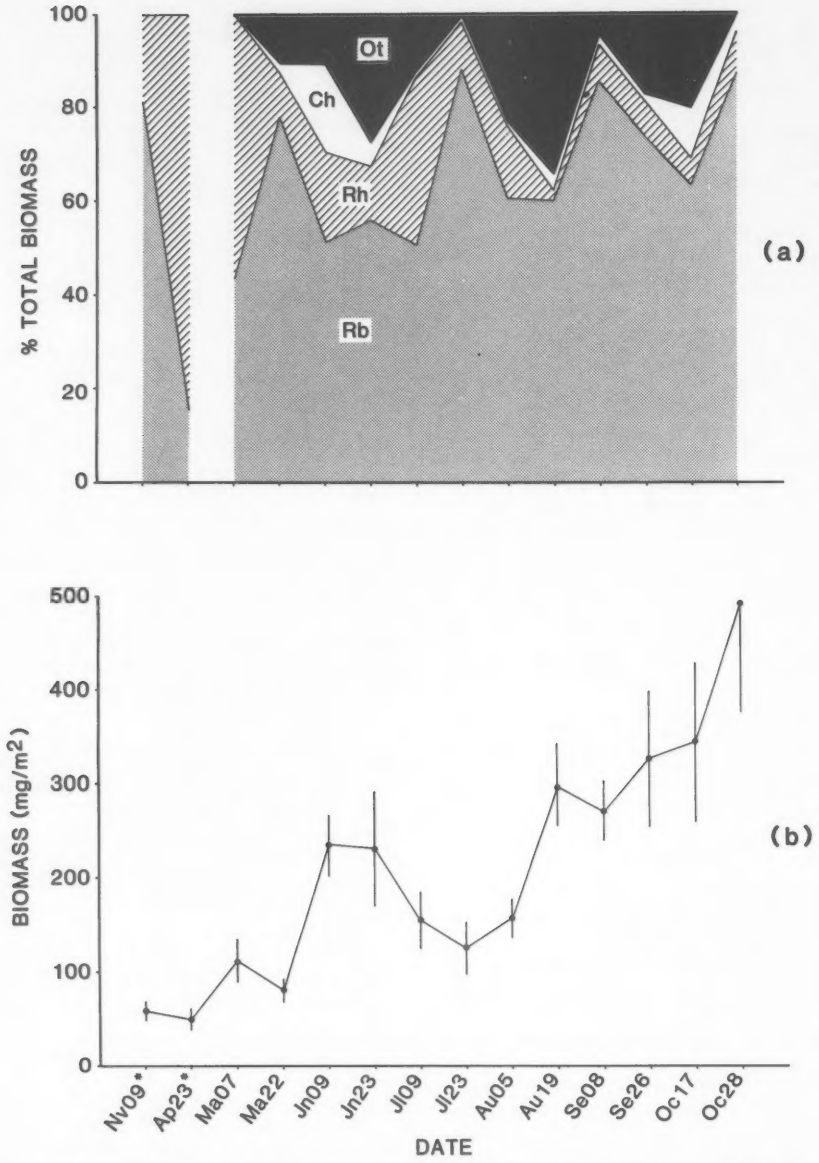

FIG. 1. (a) Contribution of *R. demeijerei* (Rb), *Rheosmittia* sp. (Rh), other chironomids (Ch), and other organisms (Ot) to total dry biomass; (b) mean dry mass (±SE) of all macroinvertebrates in shifting sand areas of the Sand River. Asterisks indicate dates where biomass was determined only for *R. demeijerei* and *Rheosmittia* sp.

11 964 and 77 625 individuals/m^2. Biomass was slightly overestimated because sphaeriid clams, which occurred in significant numbers in this area, were weighed in their shells. Larvae of *R. demeijerei* and *Rheosmittia* sp. consistently contributed the majority of the chironomid (mean = 94.6%, SE = 2.3) and total invertebrate biomass (mean = 80.6%, SE = 3.7) (Fig. 1a). They were also the numerically dominant taxa (Fig. 2a), contributing a mean of 92.8% (SE = 1.3) of the total number of invertebrates.

Most macroinvertebrates occurred in the upper 10 cm of substrate, and none were found below 20 cm (Fig. 3). Changes in the distribution of the macroinvertebrates during the freezing process are unlikely (Pugsley and Hynes 1983).

Rheosmittia sp. exhibited a bivoltine cycle (Fig. 4), with a short summer generation (hatches in early June) and a long overwintering generation (hatches in late August). The life cycle of *R. demeijerei* (Fig. 4) is less clear, probably because larvae overwinter in both second and third instar. The pattern of relative abundances of first and second instar larvae suggests a

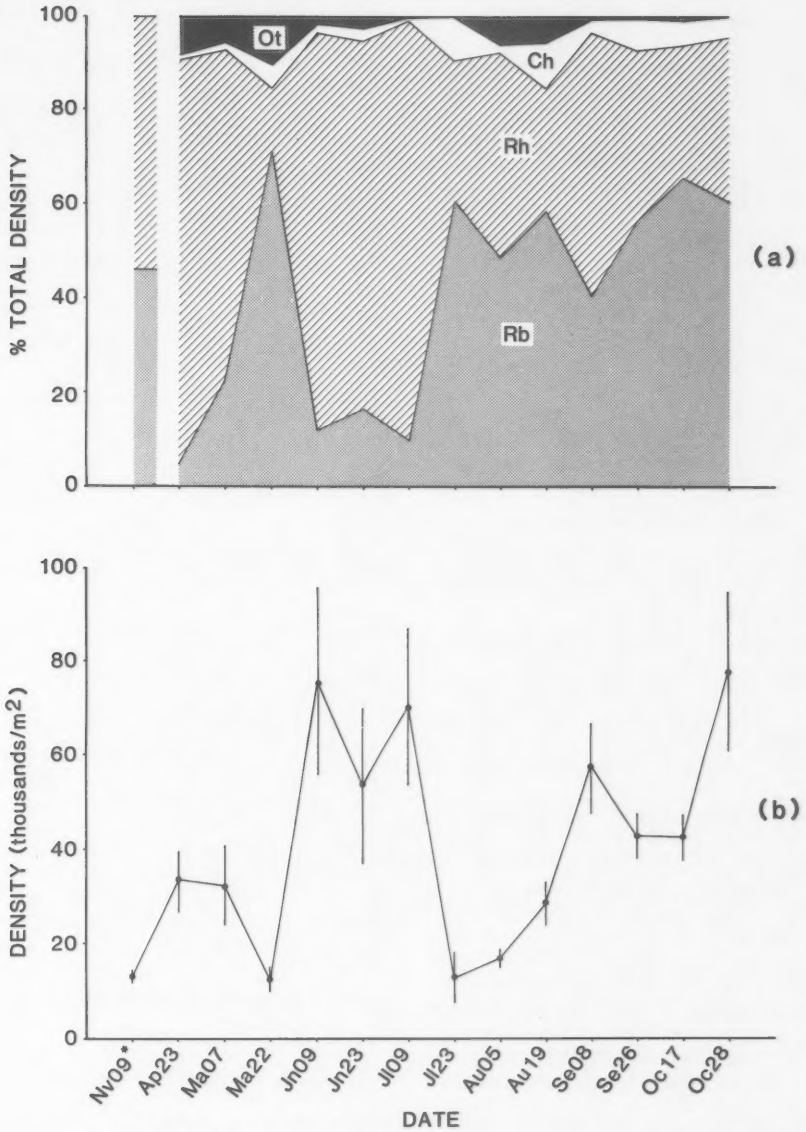

FIG. 2. (a) Contribution of *R. demeijerei* (Rb), *Rheosmittia* sp. (Rh), other chironomids (Ch), and other organisms (Ot) to total density; (b) mean density (±SE) of all macroinvertebrates in shifting sand areas of the Sand River. Asterisk indicates dates where density was determined only for *R. demeijerei* and *Rheosmittia* sp.

univoltine cycle with an extended emergence period. Other aspects of the life cycle and distribution of these species are discussed in Soluk (1983).

The sum of the annual production estimates (Table 2) for *R. demeijerei* and *Rheosmittia* sp. was used as a minimum estimate of total annual macroinvertebrate production from shifting sand ($752.03 \pm 144.50\ mg \cdot m^{-2} \cdot yr^{-1}$). Annual production to mean biomass ($P/\bar{B}$) ratios were 3.7 and 7.7 for *R. demeijerei* and *Rheosmittia* sp., respectively; the combined $P/\bar{B}$ ratio was 4.4.

Guts of *Rheosmittia* sp. larvae were tightly packed with diatoms, suggesting that this species is herbivorous. Guts of *R. demeijerei* larvae, although containing some diatoms, were mostly filled with an unidentifiable amorphous material. Since many predaceous chironomids do not consume the hard parts of their prey (Oliver 1971), it was impossible to determine whether *R. demeijerei* larvae are predators or detritivores.

Discussion

Sampling Considerations

Although total benthic macroinvertebrate density and bio-

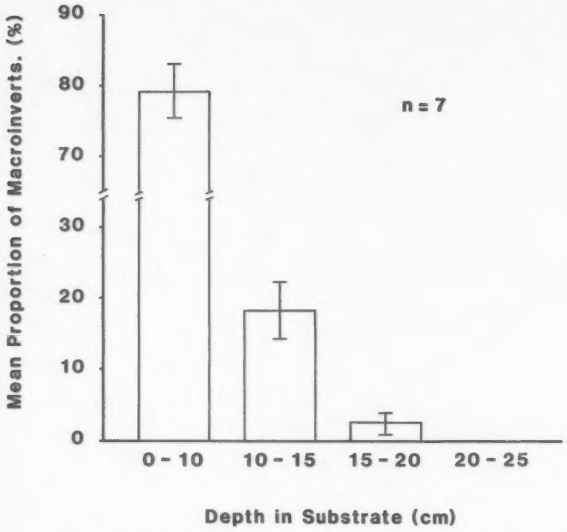

FIG. 3. Vertical distribution of macroinvertebrates in seven cores from shifting sand areas of the Sand River. Vertical bars represent 1 standard error of mean.

TABLE 2. Production (dry mass) by instar and total annual production (±95% C.I.), using the size-frequency method, for the two dominant species of Chironomidae in shifting sand areas of the Sand River, Alberta.

Instar	No./m²	Mean wt (mg)	No. loss	Wt at loss (mg)	Wt loss (mg/m²)	P (mg/m²)
			Rheosmittia sp.			
I + II	16 464	0.0007	10 674	0.0011	11.74	35.22
III	5 790	0.0017	4 308	0.0045	15.08	45.23
IV	1 482	0.0072	1 482	0.0072	10.67	32.01
Total						112.46
Annual production = 112.46 × 2 = 224.92 ± 83.47 mg·m^{-2}·yr^{-1}						
			R. demeijerei			
I	346	0.0015	−5 922	0.0023	−13.62	−54.48
II	6 268	0.0035	−939	0.0066	−6.20	−24.79
III	7 207	0.0126	5 813	0.0191	111.03	444.12
IV	1 394	0.0291	1 394	0.0291	40.56	162.26
Total						527.10
Annual production = 527.10 × 1 = 527.10 ± 61.03 mg·m^{-2}·yr^{-1}						

mass on shifting sand areas in the Sand River were generally higher than values reported in most other studies, they were comparable with values reported by Barton and Lock (1979) and Benke et al. (1984) (Table 3). Differences between this latter group of studies and the others are probably mostly attributable to sampling techniques, rather than actual differences between rivers.

Shifting sand areas are dominated by interstitial or burrowing invertebrates, which are small and difficult to detect with the unaided eye, especially in early life stages. In addition, live larvae of *R. demeijerei* and *Rheosmittia* sp. in the Sand River were able to use the copious amounts of silk they produce to retain their hold on the substrate even during vigorous elutriation of the sediments. Thus, hand-picking or live-picking techniques such as used by Berner (1951) and Monakov (1969) greatly underestimate the number of benthic macroinvertebrates.

It is significant that studies reporting the smallest mesh sizes in sample processing (0.18 mm, Barton and Lock 1979; 0.10 mm, Benke et al. 1984; 0.063 mm, this study) also reported the largest number of invertebrates in sand areas. Coarse mesh sizes (>0.50 mm) are relatively ineffective in retaining chironomid larvae and oligochaetes (Mason et al. 1975). This situation is aggravated by the minute size of most riverine psammophilous chironomid larvae (Barton 1980), which would be retained even less frequently than the generally larger forms associated with other lotic habitats. Thus, the studies that used large mesh sizes (>0.50 mm) in sample processing greatly underestimated abundance and biomass of chironomidae in shifting sands.

Barton (1980) used a relatively small mesh size in sample processing, and reported relatively low values for macroinvertebrate abundance in shifting sands (Table 3). However, macroinvertebrate density may have been underestimated, since only the top 5 cm of sand was sampled (many animals occurred below this level in my study) and an elutriation technique was used to extract live organisms from sand.

Production from Shifting Sand

Benke et al. (1984) have estimated total secondary produc-

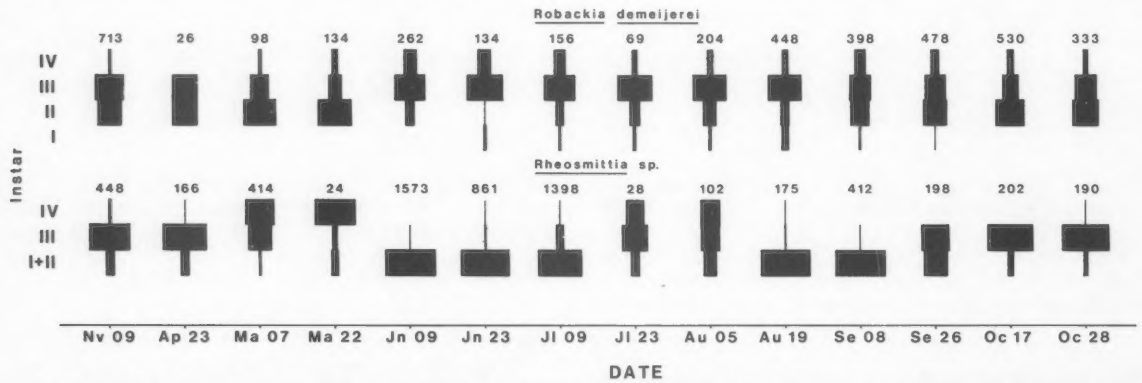

FIG. 4. Relative frequency of discernible larval instars of *R. demeijerei* and *Rheosmittia* sp. over the study period. Number of animals examined on each date is indicated above each histogram.

TABLE 3. Ranges of macroinvertebrate density and dry biomass reported from unstable sand areas in the mainstream of selected lowland rivers (NR = not reported).

River	Reference	Sampler	Mesh size (mm)	Density (no./m^2)	Biomass (mg/m^2)
Missouri	Berner 1951	Petersen grab	NR	NR	0.19–12.5[a]
White Nile	Monakov 1969	Petersen grab	NR	NR	0–200
Fraser	Northcote et al. 1976	Petersen and Ponar grabs	0.61	19–979	11–146[a]
Athabasca	Barton and Lock 1979	Ekman grab	0.18	1100–40 000	60–200
Athabasca	Barton 1980	Airlift sampler	0.20	1675–3564	NR
Mississippi	Seagle et al. 1982	Petersen grab	0.61	200–300	NR
Satilla	Benke et al. 1984	Ponar grab	0.10	20 477–49 006	93.8–156.3
Sand	This study	Core-freezer	0.06	11 964–77 625	50.0–492.1

[a]Dry mass approximations from wet mass using 0.17 as a conversion factor (Waters 1977).

tion from mainstream shifting sand areas of the Sattilla River to range between 13.7 and 28.4 $g \cdot m^{-2}$ (dry mass)$\cdot yr^{-1}$. Most of this production was contributed by the chironomids *Robackia claviger* and *Parakieferiella* (=*Rheosmittia*). Production values for *R. demeijerei* and *Rheosmittia* sp. from the Sand River were considerably lower (752.03 $mg \cdot m^{-2} \cdot yr^{-1}$). Much of the difference in values is probably attributable to differences in estimating the growth rates of psammophilous chironomid species. Mackey (1977) reported that small lotic chironomids (species not associated with shifting sand areas) had high growth rates and exhibited generation times as low as 5–8 d in warm temperatures (15–20°C) and with unlimited food supplies. By extrapolating from Mackey's data, Benke et al. (1984) estimated 18 and 46 generations per year for *R. demeijerei* and *Rheosmittia*, respectively, in the warm water (mean annual temperature 19°C) of the Sattilla River. *Rheosmittia* sp. and *R. demeijerei* in the Sand River exhibited development rates much lower than expected, given the predictions of Mackey (1977). For the 4 mo when mean temperature was 19.5°C in the Sand River, extrapolations from Mackey's data predict at least 8–12 generations for small chironomids. However, only *Rheosmittia* sp. was clearly able to complete an entire generation in this period; thus, the applicability of Mackey's chironomid growth data to species associated with shifting sand areas is doubtful. Because the Sattilla River accumulates about three times the degree-days of the Sand River, it seems likely that chironomid production will be higher in the Sattilla River; however, it is unlikely to be nearly as high as estimated by Benke et al. (1984).

The marked departure of *Rheosmittia* sp. and *R. demeijerei* from growth rates exhibited by other lotic chironomid species suggests that these species are growth limited in shifting sand areas. Actively moving sands entrain relatively little fine particulate organic material (Hynes 1970). Thus, it seems likely that food limitation may be a major constraint on collector–gatherers in such areas. However, the relationship between amounts of detritus and the dominant chironomids in shifting sand areas of the Sand River was somewhat obscure. Gut analysis indicated that *Rheosmittia* sp. fed largely on diatoms. Members of the genus *Robackia* are usually considered to be predators; however, gut content analysis did not indicate whether *R. demeijerei* was detritivorous or carnivorous. If *R. demeijerei* is predaceous, then neither of the dominant species in shifting sand areas of the Sand River exploit detritus extensively, suggesting that macroinvertebrate production in this area may be more affected by primary productivity than by allochthonous inputs.

The presence of large numbers of invertebrates, such as *Rheosmittia*, dependent upon autochthonous inputs is predicted by the river continuum concept (Vannote et al. 1980) for streams of intermediate (fourth to sixth) order, although their presence in unstable shifting sand areas is perhaps unexpected. *Rheosmittia* sp. (referred to as Orthocladiinae "B"; D. R. Oliver, pers. comm.) also occurs in high densities in the Athabasca River (Barton and Lock 1979), a much larger and generally silty stream. The diet of *Rheosmittia* sp. in the Athabasca River is unknown, but if they are diatom feeders in this system then the suggestion that primary production is relatively insignificant in such systems (Berner 1951; Barton and Lock 1979; Benke et al. 1984) may have to be reviewed.

Although food limitation seems a likely explanation for the relatively slow growth and development of *R. demeijerei* and *Rheosmittia* sp., other stresses such as displacement and reentry into the substrate, or production of large amounts of silk to maintain position in the substrate, may also impose limitations on the development rate of these chironomids.

Although larvae of *R. demeijerei* and *Rheosmittia* sp. develop slowly, this was not true of all macroinvertebrates associated with shifting sand areas of the Sand River. Larvae of *Pseudiron centralis* McDunnough prey on chironomids in shifting sand and grow faster (hatching to emergence, approximately 8 wk) than many other large mayflies (Soluk and Clifford 1984, 1985).

Importance of Shifting Sands

Although shifting sand areas support relatively little macroinvertebrate biomass or production on a per unit area basis when compared with other types of lotic habitats (e.g. MacFarlane and Waters 1982; Benke et al. 1984), they are significant in the river as a whole. Published estimates of the proportion of lowland river beds composed of unstable sandy sediments range from 70–80% for the Satilla River (Benke et al. 1984) to 90–95% for some rivers in the USSR (Zhadin and Gerd 1961). A large proportion of these sandy areas would fall under my narrower definition of shifting sand substrate. A conservative estimate for the relative proportion of the river bed composed of

shifting sand in the lower reaches of the Sand River is 80% (based on aerial photographs and surface observations).

Production estimates for other habitats (e.g. snags, silt areas, gravel bars) were not obtained in the Sand River; however, Benke et al. (1984), after comparing several habitats, considered mainstream sand areas to be the major source of macroinvertebrate production in the Satilla River. Although their estimate of production may have overemphasized the relative importance of this habitat, it still seems clear that such areas make significant contributions to macroinvertebrate production in rivers, and should be considered in studies of these systems.

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References

BARTON, D. E. 1980. Benthic macroinvertebrate communities of the Athabasca River near Ft. Mackay, Alberta. Hydrobiologia 74: 151–160.

BARTON, D. E., AND M. A. LOCK. 1979. Numerical abundance and biomass of bacteria, algae and macrobenthos of a large northern river, the Athabasca. Int. Rev. Gesamten Hydrobiol. 64: 345–359.

BENKE, A. C., T. C. VAN ARSDALL, JR., D. M. GILLESPIE, AND F. K. PARRISH. 1984. Invertebrate productivity in a subtropical blackwater river: the importance of habitat and life history. Ecol. Monogr. 54: 25–63.

BERNER, L. M. 1951. Limnology of the lower Missouri River. Ecology 32: 1–12.

HAMILTON, A. L. 1969. On estimating annual production. Limnol. Oceanogr. 14: 771–782.

HYNES, H. B. N. 1970. The ecology of running waters. University of Toronto Press, Downsview, Ont. 555 p.

KRUEGER, C. C., AND F. B. MARTIN. 1980. Computation of confidence intervals for the size-frequency (Hynes) method of estimating secondary production. Limnol. Oceanogr. 25: 773–777.

LENAT, D. R., D. L. PENROSE, AND K. W. EAGLESON. 1981. Variable effects of sediment addition on stream benthos. Hydrobiologia 79: 187–194.

LEOPOLD, L. B., M. G. WOLMAN, AND J. P. MILLER. 1964. Fluvial processes in geomorphology. W. H. Freeman, San Francisco, CA. 522 p.

MACFARLANE, M. B., AND T. F. WATERS. 1982. Annual production by caddisflies and mayflies in a western Minnesota plains stream. Can. J. Fish. Aquat. Sci. 39: 1628–1635.

MACKEY, A. P. 1977. The growth and development of larval Chironomidae (Diptera). Oikos 28: 270–275.

MASON, W. T. JR., P. A. LEWIS, AND P. L. HUDSON. 1975. The influence of sieve mesh size selectivity on benthic invertebrate indices of eutrophication. Int. Ver. Limnol. Verh. 19: 1550–1561.

MONAKOV, A. V. 1969. The zooplankton and zoobenthos of the White Nile and adjoining waters in the Republic of Sudan. Hydrobiologia 33: 161–185.

NIEWESTNOVA-SHADINA, K. 1935. Zur Kenntnis des rheophilen Mikrobenthos. Arch. Hydrobiol. 28: 555–582.

NORTHCOTE, T. G., N. T. JOHNSTON, AND K. TSUMURA. 1976. Benthic, epibenthic and drift fauna of the Lower Fraser River. Technical Report 11, Westwater Research Institute, University of British Columbia, Vancouver, B.C. 227 p.

NUTTALL, P. M. 1972. The effects of sand deposition upon the macroinvertebrate fauna of the River Camel, Cornwall. Freshwater Biol. 2: 181–186.

OLIVER, D. R. 1971. Life history of the Chironomidae. Annu. Rev. Entomol. 16: 211–230.

PUGSLEY, C. W., AND H. B. N. HYNES. 1983. A modified freeze-core technique to quantify the depth distribution of fauna in stony streambeds. Can. J. Fish. Aquat. Sci. 40: 637–643.

SEAGLE, H. H., J. C. HUTTON, AND K. S. LUBINSKI. 1982. A comparison of benthic invertebrate community composition in the Mississippi and Illinois Rivers, Pool 26. J. Freshwater Ecol. 1: 637–650.

SHAPIRO, J. 1958. The core freezer: a new sampler for lake sediments. Ecology 39: 758.

SIOLI, H. 1975. Tropical river: the Amazon, p. 461–488. *In* B. A. Whitton [ed.] River ecology. University of California Press, San Francisco, CA.

SOLUK, D. A. 1983. Ecology of shifting sand areas in rivers. M.Sc. thesis, University of Alberta, Edmonton, Alta. 120 p.

SOLUK, D. A., AND H. F. CLIFFORD. 1984. Life history and abundance of the predaceous psammophilous mayfly *Pseudiron centralis* McDunnough (Ephemeroptera: Heptageniidae). Can. J. Zool. 62: 1534–1539.

1985. Microhabitat shifts and substrate selection by the psammophilous predator *Pseudiron centralis* McDunnough (Ephemeroptera: Heptageniidae). Can. J. Zool. 63. (In press)

VANNOTE, R. L., G. W. MINSHALL, K. W. CUMMINS, J. R. SEDELL, AND C. E. CUSHING. 1980. The river continuum concept. Can. J. Fish. Aquat. Sci. 37: 130–137.

WATERS, T. F. 1977. Secondary production in inland waters. Adv. Ecol. Res. 10: 91–164.

WATERS, T. F., AND G. W. CRAWFORD. 1973. Annual production of a stream mayfly population: a comparison of methods. Limnol. Oceanogr. 18: 289–296.

ZHADIN, V. I., AND S. V. GERD. 1961. Fauna and flora of the rivers, lakes and reservoirs of the U.S.S.R. Smithsonian Institution Press, Washington, DC. 626 p. (Translated by Israel Program for Scientific Translations)

Nutrient Dynamics in a Littoral Sediment Colonized by the Submersed Macrophyte *Myriophyllum spicatum*

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Carignan, R. 1985. Nutrient dynamics in a littoral sediment colonized by the submersed macrophyte *Myriophyllum spicatum*. Can. J. Fish. Aquat. Sci. 42: 1303–1311.

The distributions of porewater reactive phosphorus (RP), NH_4^+, K, and inorganic carbon (ΣCO_2) are compared for sediments colonized by *Myriophyllum spicatum* (9–40 $g \cdot m^{-2}$) and sediments experimentally maintained plant-free. Porewater nutrients are characterized by a high spatial and temporal variability. During the summer months, root activity induces marked reductions in porewater RP and NH_4^+ between 5 and 38 cm. However, this trend is reversed in spring and fall when higher nutrient concentrations are observed in the colonized sediments, presumably as a result of root decay. Root activity is also associated with higher ΣCO_2 and K in the porewaters. Exchangeable NH_4^+ is the largest pool of available N and exhibits a relatively rapid (9.5–24 d) turnover time in colonized sediments. The mineralization of organic matter is an important source of sediment NH_4^+ and comparable in quantity with the N requirement of the macrophytes. These results suggest that under conditions of nutrient limitation, the rate of nutrient production from sedimentary organic matter decomposition may be a good predictor of macrophyte growth.

On compare la distribution du phosphore réactif (PR), du NH_4^+, du K et du carbone inorganique (ΣCO_2) dans les eaux interstitielles de sédiments colonisés par *Myriophyllum spicatum* (de 9 à 40 $g \cdot m^{-2}$) et de sédiments libres de croissance végétale. Les bioéléments dissous dans ces eaux sont caractérisés par une variabilité spatiale et temporelle élevée. Pendant l'été, la croissance des racines entraîne des baisses marquées du PR et du NH_4^+ entre 5 et 38 cm. Toutefois, cette tendance est inversée au printemps et en automne quand des concentrations plus élevées de bioéléments sont présentes dans les sédiments colonisés, probablement à la suite de la décomposition des racines. La croissance des racines correspond aussi à une hausse de ΣCO_2 et de K dans les eaux interstitielles. Le NH_4^+ échangeable, qui représente le plus important réservoir de N disponible, se renouvelle assez rapidement (9,5–24 jours) dans les sédiments colonisés. La minéralisation de matières organiques constitue une importante source de NH_4^+ sédimentaire qui suffit à répondre aux besoins des macrophytes en N. Les résultats portent à croire que le taux de production de bioéléments par décomposition de matières organiques sédimentaires pourrait être un bon indice de la croissance des macrophytes quand la quantité de bioéléments est limitée.

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Much attention has recently been given to the impact of aquatic macrophytes on their surrounding environment. Macrophytes can contribute significantly to the primary production of shallow lakes (Wetzel 1975). They release significant amounts of nutrients (Carpenter 1980) and organic compounds (Godshalk and Wetzel 1976), particularly at die-off. Aquatic macrophytes also harbor an active epiphitic community (Jones 1980) that can account for much of the primary production occurring within a weedbed (Cattaneo and Kalff 1980). However, little is known about the effects of rooted macrophytes on their substrate. Possible effects could be physical, such as the alteration of local sedimentation patterns in the littoral zone, or chemical, through the alteration of sediment E_h and pH (Tessenow and Baynes 1978) or absorption of nutrients and release of organic compounds by root exudation and turnover.

The sediment-related properties controlling the presence and abundance of macrophytes are also poorly understood. It is widely accepted that rooted macrophytes rely principally on sediments for N and P supply (Nichols and Keeney 1976; Smith 1978; Carignan 1982), and that sediment nutrient availability can affect growth (Barko and Smart 1979). However, predictive models relating specific sediment properties to macrophyte production or abundance still remain to be formulated. Because nutrient availability and supply have often been shown to control growth and biomass in aquatic systems, these factors should also be taken into consideration in the development of models designed to predict macrophyte biomass and production. An obvious preliminary step to the development of such models is the identification of the principal sedimentary pools and reactions capable of influencing nutrient availability to rooted macrophytes. The relative importance of potential nutrient pools (exchangeable, dissolved, labile organic or inorganic) or sources (mineralization, dissolution of mineral phases, groundwater advection) should therefore be established for a variety of littoral environments in order to isolate the most important ones. The seasonal evolution of these pools and sources should also be explored in order to minimize the effects of the seasonal variability of model inputs. With these objectives in mind, the present study was designed to evaluate the

effects of macrophytes on some aspects of the seasonal nutrient dynamics in sediments and to identify the principal sources of available nutrients in littoral sediments.

Methods

Study Area

The study was conducted in a protected embayment (Quinn Bay) of Lake Memphremagog (Quebec–Vermont, 45°06′N, 72°16′W). The lake is mesotrophic and is surrounded by a hilly metamorphic bedrock mainly composed of slate, sandstone, and limestone (Boucot and Drapeau 1968), overlain by a variable thickness of glacial till. The shoreline (1.5 km) of Quinn Bay is relatively undisturbed, and most of its watershed (approximately 1 km^2) is occupied by a mature stand of mixed hardwoods dominated by sugar maple (*Acer saccharum*) and yellow birch (*Betula lutea*). The area supports one permanent dwelling and four summer cottages. The closest habitation lies approximately 400 m from the study site. The submerged vascular flora has been dominated by *Myriophyllum spicatum* (20–60 $g \cdot m^{-2}$) at least since 1975. Other species include *Vallisneria americana* (2–5 $g \cdot m^{-2}$), *Heteranthera dubia* (0–5 $g \cdot m^{-2}$), *Potamogeton richardsonii* (0–5 $g \cdot m^{-2}$), and *P. robbinsii* (0–5 $g \cdot m^{-2}$).

Experimental Design

To investigate the effects of macrophyte presence or absence on sediment properties, eight identical quadrats (area: 4.0 m^2) were positioned in a relatively homogenous part of an extensive *Myriophyllum* bed (8–12 $g \cdot m^{-2}$ dry wt^{-1}, spring biomass). The depth of the quadrats varied from 1.8 to 2.2 m. At the beginning of the growth season (June 1, 1979), SCUBA divers harvested all plants from four of the quadrats by cutting the stems at the sediment–water interface. The four remaining quadrats were left intact. The harvested quadrats were maintained plant-free for a period of 14 mo. Very little regrowth occurred in the harvested quadrats; the few appearing plants consisted mainly of *V. americana*, *H. dubia*, and *M. spicatum* and were removed during weekly visits. The aboveground plant biomass occurring near the experimental quadrats was measured on one occasion (September 1979) by harvesting 0.79-m^2 circular areas positioned randomly within 3 m of the quadrats.

The sediment porewater was sampled with close-interval Plexiglas dialyzers (Hesslein 1976) fitted with biologically inert membranes (Gelman DM-450 or HT-450). Cellulose-based membranes were found to be useless because a bacterial attack of the membranes induced a decrease in porewater nutrients and a simultaneous increase in metabolites such as CH_4 and ΣCO_2 (Carignan 1984). The samplers were inserted randomly within the quadrats and left for 10–15 d before retrieval. Preliminary tests indicated that the empirical equilibration time of the samplers in these particular sediments is about 6 d for NH_4^+ and 10 d for reactive phosphorus (RP). The samplers were sampled at 2-cm intervals within 5 min following removal from the sediments; more prolonged delays caused significant losses of RP at this particular site. The samples were injected into polystyrene vials preacidified with 30 μL of H_2SO_4 (1 $mol \cdot L^{-1}$) and stored at 4°C until needed. Aliquots were analyzed for NH_4^+ and RP within 2 d by automated methods. Nitrate + nitrite were also measured monthly in the water column. All wet analytical methods used in this study were taken from Stainton et al. (1977). Sediment E_h and pH were measured in colonized sediments only by direct insertion of combination probes in freshly cored sediments; the E_h values are reported relative to the standard hydrogen electrode.

All sediment cores were obtained with a Brown's piston corer and extruded within 6 h. Root biomass was measured by washing fresh 5-cm sediment sections on a 500-μm sieve and drying the root fragments overnight at 65°C. Exchangeable NH_4^+ was measured on sediment subsamples within 2 h of collection. The extraction was performed by periodic agitation of 5–10 g (fresh weight) of sediment in KCl (2 $mol \cdot L^{-1}$) for 1 h, followed by steam distillation and colorimetric determination of NH_4^+. Core sections to be preserved for subsequent analyses were stored in plastic vials at −20°C. Total C and N were measured with a Hewlett-Packard CHN analyzer. Total P was measured after a $HClO_4$–HNO_3 digestion (Sommers and Nelson 1972). Because some sediment horizons contained substantial amounts of pebbles and wood debris, total C, N, and P analyses were performed on the <1 mm fraction.

The rates of NH_4^+ production by mineralization were calculated from the changes in exchangeable NH_4^+ content of homogenized fresh sediment sections incubated under anaerobic conditions. Aliquots (10–20 g) of unsorted sediments were introduced into 50-mL Erlenmeyer flasks to which 20 mL of distilled water was added to facilitate homogenization upon subsampling. The samples were then agitated for 10–20 s with a magnetic stirrer and the resulting suspension subsampled with a 5-mL automatic pipette during agitation. The flasks were then capped with a rubber septum and bubbled for 10 min with N_2 through syringe needles. After 15 d of incubation at 20°C in a N_2-filled glove box, the flasks were agitated and subsampled again. The exchangeable NH_4^+ was extracted from the sediment suspensions as described above and the rates of NH_4^+ production were calculated from the difference in total NH_4^+ content over time in the flasks. It should be noted that the possible physical breakdown of natural sediment aggregates resulting from the agitation step may have stimulated NH_4^+ production in this procedure. The rates of NH_4^+ production may therefore have been overestimated in this study.

Groundwater advection was measured with flowmeters (Lee 1977) consisting of inverted barrel sections (height: 30 cm, area: 2820 cm^2) to which collapsible sampling bags were attached. Flowmeters were installed near each of the eight quadrats and were visited weekly, or more often during periods of heavy rainfall. Precipitation data were obtained for the town of Magog (15 km north of the study site) from Environment Canada.

Results and Discussion

Sediment Composition

During the 1979 and 1980 growth seasons, both colonized and uncolonized sediments exhibited reducing conditions, as indicated by the presence of detectable H_2S and CH_4 from 2 cm downwards (LaZerte 1981). The physical and chemical characteristics of the sediments are presented in Table 1. The mean sediment porosity and total C, N, and P were not significantly different (*t*-test, $p < 0.05$) for colonized and uncolonized sediments and are grouped together in the table. The vertical structure of the sediments is composed of a superficial layer (0–16 cm) of sandy silt, relatively rich in fine organic matter, underlain by a second layer characterized by an abundance of wood debris (1–50 mm in diameter) and pebbles (1–5 mm in diameter). The mean ($n = 6$) C, N, and P content of the wood

TABLE 1. Mean composition and physical and chemical properties ±1 SE (n = 8) of the sediments studied. pH and E_h were measured on a single core collected in July 1980 in a colonized quadrat.

Depth (cm)	Porosity (%)	Fraction > 1 mm (% of dry wt)		Total C (% of dry wt)	Total N (% of dry wt)	Total P ($\mu g \cdot g^{-1}$)	pH	E_h (mV)
		Wood	Pebbles					
2–4	89.0±0.5	3.0±1.7	0.1±0.1	7.57±0.67	0.56±0.04	757±36	6.86	+36
6–8	87.2±0.7	1.9±1.2	0.1±0.1	7.81±0.50	0.55±0.03	771±28	6.75	−82
10–12	85.8±0.7	1.0±0.2	0.0	7.19±0.36	0.48±0.02	725±37	6.71	−117
14–16	85.4±0.2	2.4±0.7	0.7±0.6	5.66±0.33	0.37±0.03	636±55	6.66	−114
18–20	82.4±1.5	5.8±2.3	1.8±1.2	5.14±0.66	0.32±0.04	497±37	6.64	−115
22–24	77.3±2.2	21.3±8.7	3.6±1.8	5.42±1.01	0.34±0.09	457±44	6.63	−115
26–28	71.5±1.4	11.1±4.4	4.0±1.9	5.30±1.09	0.26±0.05	408±34	6.61	−119
30–32	71.2±4.6	13.7±5.3	1.1±0.3	4.34±0.64	0.20±0.03	372±22	6.59	−118
34–36	66.2±1.2	10.8±0.8	0.7±0.2	7.02±1.62	0.25±0.06	385±16	—	—
38–40	—	7.6±1.5	4.2±1.3	3.75±1.08	0.13±0.03	325±19	—	—

debris present between 18 and 40 cm was 46.3 ± 1.6%, 0.71 ± 0.10%, and 0.19 ± 0.02%, respectively. The absence of coarse material in the upper 18 cm may reflect a decrease in turbulence at the time of deposition resulting from a past change in water level; it may also reflect a progressive increase in submerged macrophyte biomass following the apparition of more eutrophic conditions in the lake during the last few decades. When macrophytes are absent, fine particulate matter normally does not accumulate in the turbulent littoral zone of relatively deep lakes (Evans and Rigler 1980; Davis and Ford 1982). However, the turbulence dampening effect of macrophyte beds should favor the deposition of fine particulate matter as observed by de March (1978) for submerged mosses in an arctic lake.

Porewater Nutrients

The seasonal variations in porewater RP and NH_4^+ in colonized and uncolonized quadrats are shown in Fig. 1. With the exception of June 10, 1979, shortly after the beginning of the experiment, the mean porewater NH_4^+ concentrations of colonized and uncolonized sediments showed significant differences (t-test, $p < 0.05$). These differences were generally less marked for porewater RP, suggesting that the porewater RP is probably buffered by a relatively larger exchangeable pool than NH_4^+ is in these sediments. This suggestion is supported by an observed exchangeable/porewater ratio of 6–9:1 for NH_4^+ (see Fig. 1 and 4) compared with a mobile P/porewater RP ratio of 30–50:1 measured by isotopic dilution (Carignan and Flett 1981) in a nearby core obtained at a depth of 2.5 m.

During the aestival period, lower nutrient concentrations were observed in the colonized sediments between 5 and 38 cm; these lower concentrations apparently reflect the zone of root influence for *M. spicatum*. The root biomass profile presented in Table 2 confirms that its root system can extend as far down as 40 cm below the interface at this site. During the growth period, the low RP concentrations present in this zone are potentially limiting plant growth as shown by Smith (1978), who found a saturation concentration of 17 $\mu mol \cdot L^{-1}$ for P uptake by the roots of *M. spicatum*.

In the spring and fall, pronounced nutrient peaks appeared between 5–15 and 25–40 cm in the colonized sediments. Because similar peaks did not occur in the uncolonized sediments, they were most likely produced by the decomposition of a portion of the root system of *M. spicatum* at the end of the growth season. The reappearance of similar peaks during the following spring indicates that root decay was incomplete at the onset of winter and continued in late spring upon sediment warm-up. This root decay phenomenon is consistent with the observed annual senescence of about 75% of the aboveground component of the *M. spicatum* standing stock in wintertime at this site. The observation of spring and fall nutrient maxima in the colonized sediments has an important implication for the conception or interpretation of eventual relationships between available nutrient concentrations in the sediments and macrophyte growth or biomass: because nutrients such as N or P are often limiting in aquatic environments, it might be tempting to interpret positive empirical relationships between porewater nutrients and macrophyte biomass as an indication of nutrient limitation. The above results show that such may not be the case at least for *M. spicatum* during spring and fall, since the relatively high porewater nutrients observed during these periods appear to result from the decomposing root biomass and do not necessarily reflect nutrient availability during the growth period. In consequence, the highly dynamic nature of the seasonal evolution of porewater nutrient concentrations should be verified for other sites and taken into consideration when elaborating nutrient–biomass models or interpreting them.

The profiles obtained in late winter illustrate another important aspect of the seasonal dynamics of porewater nutrients in the littoral zone. The nutrient peaks observed in the colonized sediments during the previous fall have disappeared and have been replaced by relatively low and constant concentrations with depth. At this time of the year, similar concentration profiles are observed for colonized and uncolonized sediments. The hivernal decrease in porewater nutrients is attributable to decreased mineralization rates at lower temperatures and to the diffusive loss of nutrients to the overlying water, as observed by Klump and Martens (1981) in a marine sediment subject to seasonal temperature variations. The upward advection of groundwater during autumnal rainfalls and spring thaw (see below) also contributes to the reduction in porewater nutrients. As a result, the nutrients originating from root decay during the previous fall are not conserved in the sediments and the initial spring nutrient profiles are similar for colonized and uncolonized sediments, irrespective of their previous history. For this type of regime, the groundwater nutrient concentration thus appears to be an important factor controlling the initial spring nutrient levels in the sediments.

Porewater K and inorganic carbon (ΣCO_2) concentration profiles are presented in Fig. 2 and 3 for August 1979. In

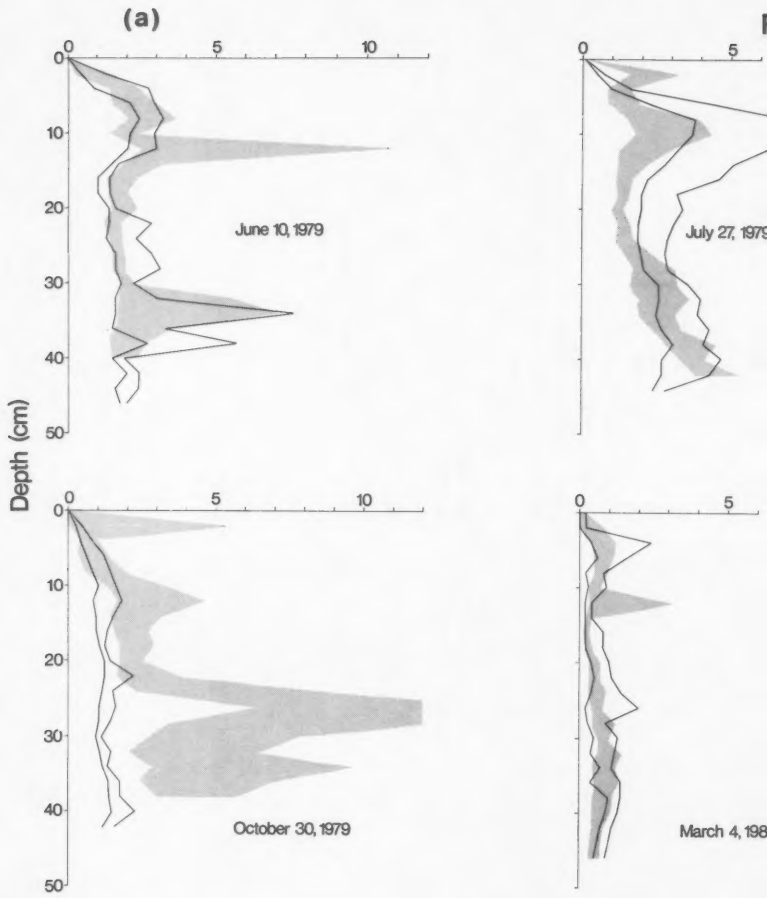
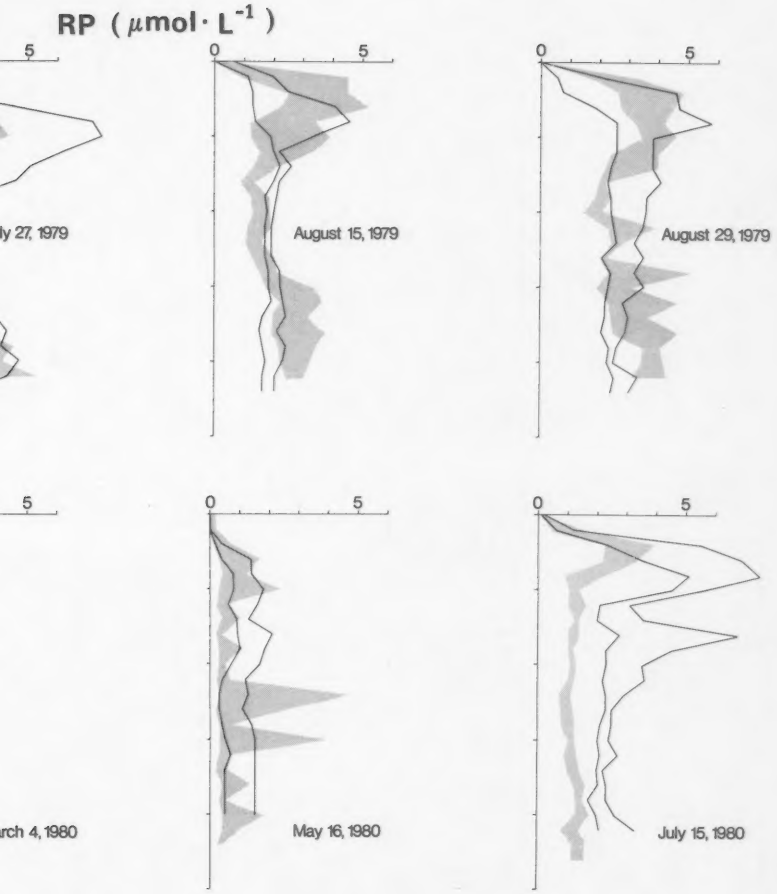


FIG. 1. Seasonal evolution of porewater (a) reactive phosphorus (RP) and (b) NH_4^+ in a littoral se represent ±1 SE of the mean, centered on the mean concentration of four replicate profiles.



ittoral sediment colonized by *Myriophyllum* (shaded areas) and uncolonized (open areas). The areas

(b)
Depth (cm)
0
50
100
10
20
30
40
June 10, 1979
October 30, 1979
March 4, 1980

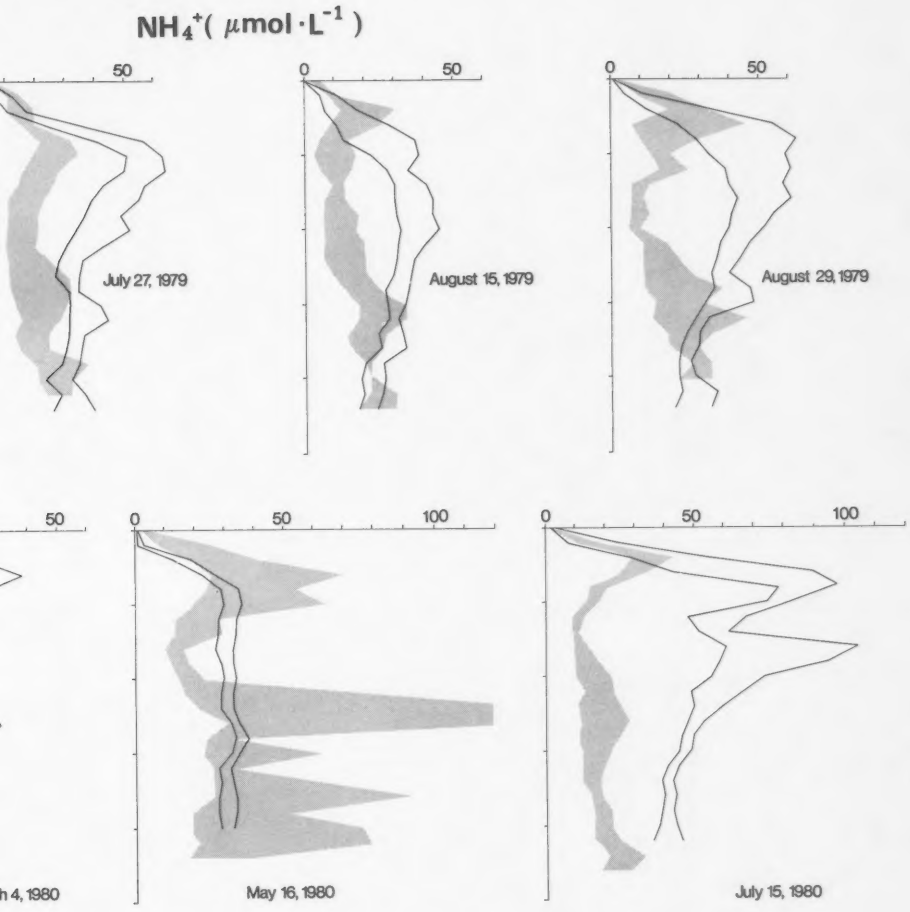


FIG. 1. (*Concluded*)

TABLE 2. Root biomass distribution with depth in colonized sediments (August 30, 1980).

Depth (cm)	Root biomass ($g \cdot m^{-2}$) Mean	SE
0–5	0.4	0.4
5–10	1.8	1.5
10–15	0.6	0.4
15–20	0.8	0.4
20–25	0.3	0.4
25–30	0.8	1.2
30–35	0.7	0.7
35–40	0.5	0.6
40–45	0.3	0.4

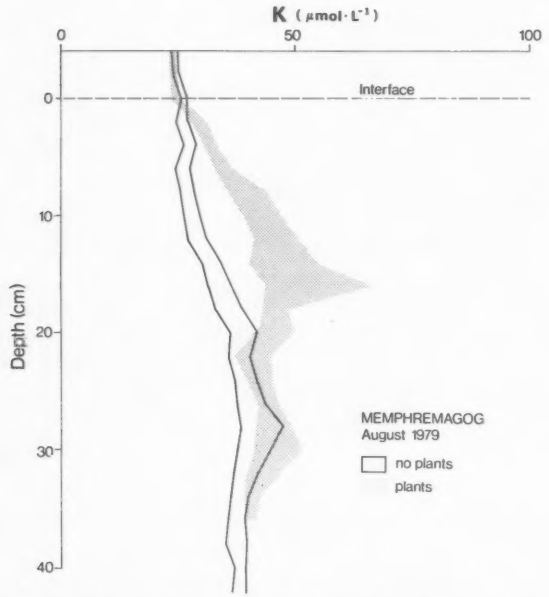


FIG. 2. Porewater K in colonized and uncolonized sediments. See legend to Fig. 1 for details.

contrast with the results obtained for RP and NH_4^+, higher K and CO_2 were observed in the colonized sediments. The higher K concentrations were somewhat unexpected, since other submersed macrophytes have been shown to meet at least some of their K requirement through root uptake (Barko 1982; Waisel et al. 1982). Macrophytes should thus constitute a sink for porewater K, and not a source as apparently observed here. The fact that the highest K concentrations coincide with the zone where NH_4^+ depletion is most pronounced in the colonized sediments indicates that the release of K is associated with root metabolism. These high K levels may be explained by a cation exchange reaction where exchangeable K is displaced from adsorption sites by another cation produced by root metabolism. Hydrogen ion is likely to be the displacing cation for the following reason: Fig. 3 shows that a substantial amount of CO_2, presumably arising from root respiration and turnover, is

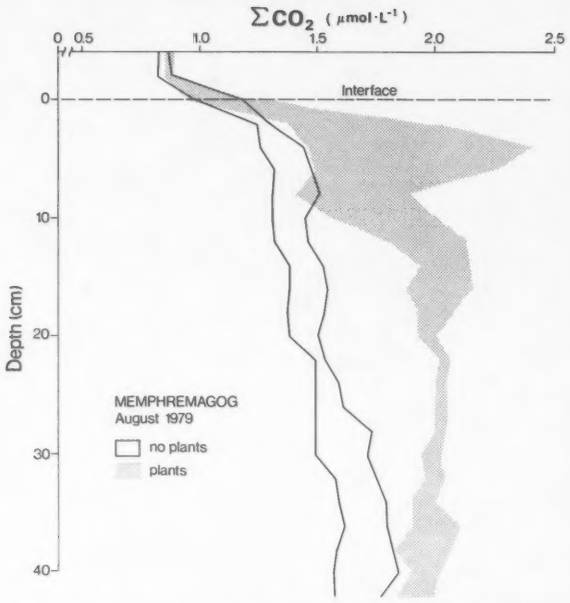


FIG. 3. Porewater dissolved inorganic carbon (ΣCO_2) in colonized and uncolonized sediments. See legend to Fig. 1 for details.

released into the porewater of colonized sediments. At the pH values reported in Table 1 for these sediments, more than half of the respired CO_2 will dissociate into H^+ and HCO_3^- (pK_1 H_2CO_3 = 6.4 at 15°C), thus providing a major source of H^+ available for exchange reactions. At this study site, it has been observed that the root system of *M. spicatum* can lower the sediment pH by approximately 0.3 unit (B. D. LaZerte, Ontario Ministry of the Environment, Dorset, Ont., pers. comm.). Another possible source of H^+ in the porewater of colonized sediments is the exchange of NH_4^+ for H^+ by the root system. Figures 1 and 4 show that NH_4^+ is assimilated in relatively large quantities by the roots. However, to maintain their cation–anion balance, the roots must release equivalent amounts of other cations. In terrestrial plants, NH_4^+ uptake is balanced by an equivalent excretion of H^+ by the roots (Kirkby 1969; Epstein 1972). Similarly, ammonium assimilation by *M. spicatum* probably results in the liberation of a significant amount of H^+ in the sediments.

Exchangeable NH_4^+

Profiles of exchangeable NH_4^+ (as NH_4-N) were obtained at the end of the 1979 growth season from colonized and uncolonized sediments and are presented in Fig. 4. The most pronounced differences occurred between 5 and 20 cm. This interval probably represents the zone of maximum exploitation by *Myriophyllum* roots as also indicated by the porewater profiles. At this time, 30.8 ± 5.9 and 57.8 ± 4.1 mmol exchangeable $NH_4^+ \cdot m^{-2}$ was present between 5 and 38 cm in the colonized and uncolonized sediments, respectively.

Groundwater

In order to estimate the contribution of groundwater in nutrient supply to macrophytes, groundwater flow rates were measured from July 26 to October 1, 1979. Groundwater flow

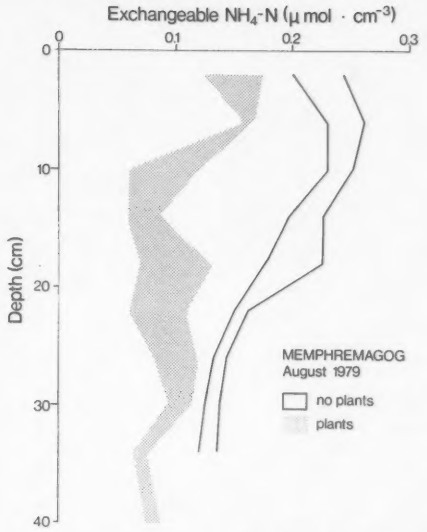


FIG. 4. Exchangeable NH_4-N in colonized and uncolonized sediments. See legend to Fig. 1 for details.

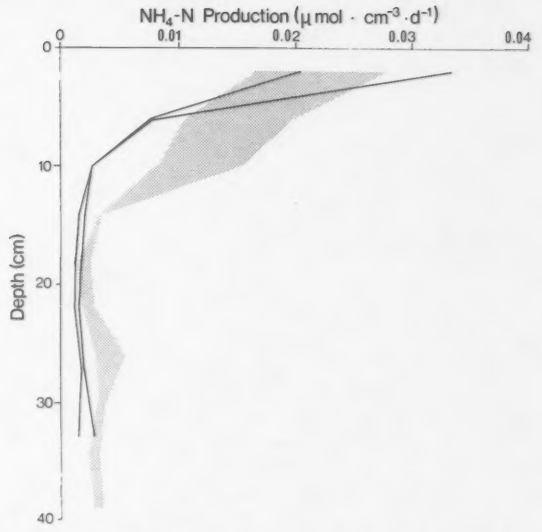


FIG. 5. Mean ± 1 SE ($n = 3$) NH_4-N production in colonized (shaded) and uncolonized (open) sediments.

was significantly correlated with rainfall ($y = 0.09x + 0.15$, $r^2 = 0.75$, $p < 0.05$). This relation was then used with local precipitation data to estimate the total amount of groundwater ($61\,L \cdot m^{-2}$) that flowed through the root zone (5–38 cm) during the 1979 growth period, assumed to extend from June 1 to October 15. When the mean porewater concentrations of RP and NH_4^+ below 38 cm are multiplied by the total groundwater flow, it is found that advection supplied 0.17 mmol $RP \cdot m^{-2}$ and 1.8 mmol $NH_4^+ \cdot m^{-2}$ to the lower boundary of the root zone. When the same procedure is used to estimate the amount of porewater nutrients leaving the upper limit of the root zone, the net contribution of groundwater flow in nutrient supply to the roots is reduced to approximately 0.02 mmol $RP \cdot m^{-2}$ and 0.19 mmol $NH_4^+ \cdot m^{-2}$ per growth period. On a yearly basis, however, advection probably causes a net loss of porewater nutrients, since the high nutrient levels generated during fall by root decay are washed out of the sediment column in late fall and during the spring thaw.

Mineralization

Figure 5 shows the mean rate of NH_4^+ production (as NH_4-N) for cores collected on October 25, 1979, in colonized and uncolonized sediments. At this time of the season, the mineralization rates were higher (under laboratory conditions) and more variable in the colonized sediments. The largest differences were observed between 6–14 and 24–30 cm and are consistent with the pronounced porewater NH_4^+ peaks observed on October 30 at the same depths in the colonized sediments (Fig. 1). This observation further supports the hypothesis according to which the rhizosphere of *M. spicatum* is partially decomposed at the end of the growth season. However, the zones of most active NH_4^+ production do not correspond to the zone of maximum root activity during the growth period as indicated by Fig. 1–4. This is contrary to what should be expected if the autumnal root decomposition was proportional to root density in the sediment. This discrepancy suggests that root decomposition occurs preferentially in certain parts of the rhizosphere during the autumnal senescence of *M. spicatum*. Assuming preferential decomposition, several idealized models of root activity and distribution in the sediment can be postulated to explain the present observations. For example, the preferential decomposition of the outer edge of pseudo-spherical rhizospheres centered 10–20 cm below the sediment–water interface should produce bimodal porewater and mineralization profiles. This hypothesis should be verifiable by intensive root sampling during the autumnal senescence of the plants or by non-steady-state tridimensional numerical modeling of NH_4^+ dispersion in sediments taking into account the effects of diffusion, advection, and adsorption.

In both sediments, the rate of mineralization was highest at the surface (0.024 $\mu mol \cdot cm^{-3} \cdot d^{-1}$, mean of all cores) and decreased rapidly to reach a relatively constant value (0.002–0.003 $\mu mol \cdot cm^{-3} \cdot d^{-1}$) from 10 to 40 cm. The nearly constant porewater NH_4^+ concentrations observed in the colonized sediment throughout the growth season (Fig. 1a) indicate that the mineralized NH_4^+ did not accumulate in the sediment but was continuously taken up by the roots. This approximate steady state between supply and demand implies a turnover rate of about 10 d at 5 cm, and 25 d at 38 cm for the exchangeable + porewater NH_4^+ pool of the colonized sediments.

The contribution of organic matter mineralization to the supply of NH_4^+ to the roots can be estimated for the total growth period by integrating the observed NH_4^+ production rate between 5 and 38 cm. The rates measured in October for the colonized sediments include root decomposition and are probably not representative of aestival conditions. For this reason, the uncolonized sediment data have been used instead. This assumes that the MH_4^+ production rates observed in October for the uncolonized sediments are also representative of the colonized sediment during the growth season. This assumption would be questionable in the case of macrophytes capable of supplying significant amounts of oxygen to the sediment, since the nature of the final electron acceptor (O_2, NO_3^-, SO_4^{2-}) can influence the rate of organic matter decomposition in sediments. However, at the present study site, the fact that healthy *Myriophyllum* roots are covered with a thin coating of an acid

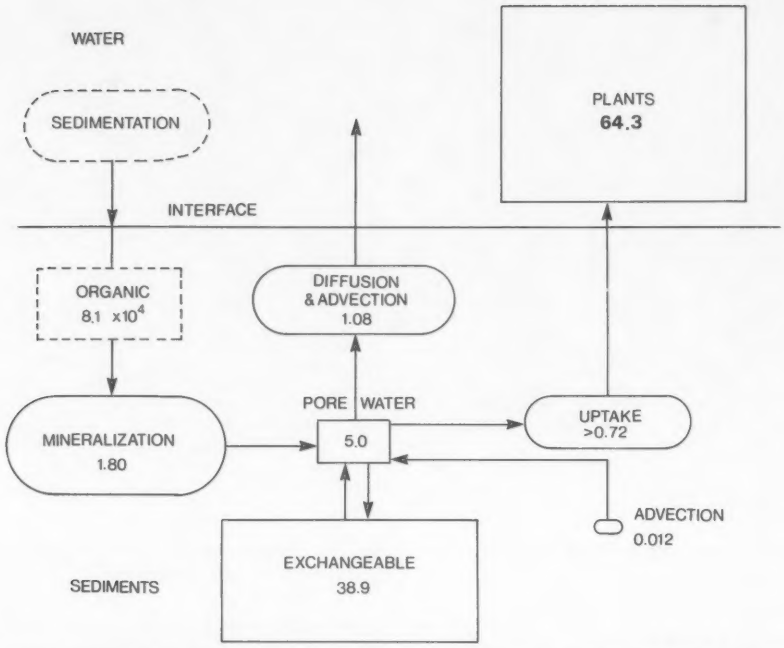


FIG. 6. Aestival N dynamics in a littoral sediment layer (0–38 cm) colonized by *Myriophyllum*. Boxes represent pools and are quantified as mmol·m^{-2} of sediment surface. Processes are represented by ovoids and their rates are expressed as mmol·m^{-2}·d^{-1}.

volatile sulfide (presumably FeS), which is unstable under oxidized conditions, suggests that root-mediated oxygen transport to the sediment is not important here. For the period extending from June 1 to September 1, the mineralization of organic matter thus supplied 0.72 mmol NH_4^+·m^{-2}·d^{-1} or 66.2 mmol NH_4^+·m^{-2} to the plants. This value is only approximate, since it is not corrected for the effect of seasonal temperature variations on N mineralization rates in these sediments. Nevertheless, it shows that mineralization is an important source of nutrients to the macrophytes of this site.

The top 5 cm accounted for 60% of the total (1.80 mmol·m^{-2}·d^{-1}) NH_4^+ production measured between 0 and 40 cm. Since the NH_4^+ produced in the 0- to 5-cm layer does not accumulate in the porewater or in the exchangeable pool, it can only be lost by diffusion and advection to the overlying water and represents a major internal nutrient loading pathway for this weed bed.

Nitrogen Budget

At the beginning of the study, the aboveground biomass (dry weight) of *M. spicatum*, collected from the cleared quadrats, was 9.0 ± 3.2 g·m^{-2} ($n = 4$). The biomass reached an apparent maximum in early September and was measured as 37.9 ± 4.6 g·m^{-2} ($n = 6$) on September 1, close to the colonized quadrats. This value is much lower than commonly reported for *M. spicatum* and suggests nutrient limitation at this site. Taking from Table 2 a value of 0.16 for below-ground/aboveground biomass ratio, approximately 33.6 plant biomass·m^{-2} accumulated during this period. When the intial and final biomasses are multiplied by their respective N content, it is found that the plants accumulated 64.3 mmol N·m^{-2}. This represents a minimum estimate of the total N demand by the plants during the growth period, since excretion and sloughing (Adams and McCracken 1974) are not taken into consideration. Because the overlying water inorganic N concentration was much lower (<1 μmol·L^{-1}) than the porewater NH_4^+ during most of the growth period, a major portion of the plant-N was probably derived from the sediment (Nichols and Keeney 1976). During the same growth period, the amount of NH_4^+ supplied to the root zone (5–38 cm) by mineralization and advection was 66.3 mmol·m^{-2} or 0.72 mmol·m^{-2}·d^{-1} (Fig. 5). This value compares well with the amount of N found in the plants at the time of maximum biomass and indicates that available N supply from the sediment may limit macrophyte production at this site. These observations suggest that the rate of nutrient supply to the sediments by organic matter mineralization may be a potentially useful predictor of macrophyte production in nutrient-limited environments.

The pools and processes most likely to influence the N distribution and dynamics in sediments colonized by aquatic macrophytes are illustrated in Fig. 6. At this particular site, diffusion/advection is an important pathway of nutrient return from the sediment to the overlying water, since most of the nutrient regeneration occurs outside (0–5 cm) the zone of root influence. The plant-mediated N translocation from the sediment to the overlying water also appears to be important in this system. It should be noted that the quantitative importance of the translocation pathway does not necessarily imply that macrophytes play an important role in the transport of N from the sediment to the overlying water. In effect, it is conceivable that in the absence of plants, the increased porewater NH_4^+ would eventually result in increased diffusive and advective losses to the overlying water. Instead, macrophytes may contribute to increase nutrient regeneration in the littoral zone by

locally enhancing the rate of labile organic matter deposition to the sediments. This "sedimentation trap" effect has been confirmed by ^{210}Pb sedimentation rate measurements (R. J. Flett, 9-30 Baylor, Winnipeg, Man., pers. comm.), indicating that in the last few decades, sedimentation has been higher at the study site ($2\,mm \cdot yr^{-1}$) than at other nearby deeper sites ($1–1.5\,mm \cdot yr^{-1}$) in Lake Memphremagog.

In nutrient-limited systems, the rate of sedimentation of labile organic matter may be an important factor controlling macrophyte biomass and productivity. At my study site, the top 5 cm of the sediment does not seem to be involved in supplying nutrients to *Myriophyllum*. Given a sedimentation rate of $2\,mm \cdot yr^{-1}$, a time lag of about 25 yr should be expected between changes in labile organic matter supply to the surficial sediments and the response of the macrophyte population.

Acknowledgements

This study was conducted while I was a Visiting Scientist at the National Water Research Institute, Burlington,, Ont. I am indebted to D. S. Painter and D. R. S. Lean for logistic support and to the The Limnology Research Center of McGill University for providing laboratory space and field facilities. I thank M. S. Adams for his constructive review of the original manuscript.

References

ADAMS, M. S., AND M. D. MCCRACKEN. 1974. Seasonal production of the *Myriophyllum* component of the littoral of Lake Wingra, Wisconsin. J. Ecol. 62: 457–465.

BARKO, J. W. 1982. Influence of potassium source (sediment vs open water) and sediment composition on the growth and composition of a submersed freshwater macrophyte (*Hydrilla verticillata* (F.f.) Royle). Aquat. Bot. 12: 157–172.

BARKO, J. W., AND R. M. SMART. 1979. The nutritional ecology of *Cyperus esculentus*, an emergent aquatic plant, grown on different sediments. Aquat. Bot. 6: 13–28.

BOUCOT, A. J., AND G. DRAPEAU. 1968. Roches siluro-dévoniennes du lac Memphrémagog et roches équivalentes dans les Canton de l'Est. Étude spéciale No. 1, Ministère des Richesses Naturelles du Québec. 45 p.

CARIGNAN, R. 1982. An empirical model to estimate the relative importance of roots in phosphorus uptake by aquatic macrophytes. Can. J. Fish. Aquat. Sci. 39: 243–247.

1984. Interstitial water sampling by dialysis: methodological notes. Limnol. Oceanogr. 29: 667–670.

CARIGNAN, R., AND R. J. FLETT. 1981. Postdepositional mobility of phosphorus in lake sediments. Limnol. Oceanogr. 26: 361–366.

CARPENTER, S. R. 1980. Enrichment of Lake Wingra, Wisconsin, by submersed macrophyte decay. Ecology 61: 1145–1155.

CATTANEO, A., AND J. KALFF. 1980. The relative contribution of aquatic macrophytes and their epiphytes to the production of macrophyte beds. Limnol. Oceanogr. 25: 280–289.

DAVIS, M. B., AND M. S. FORD. 1982. Sediment focusing in Mirror Lake, New Hampshire. Limnol. Oceanogr. 27: 137–150.

DE MARCH, L. 1978. Permanent sedimentation of nitrogen, phosphorus, and organic carbon in a high arctic lake. J. Fish. Res. Board Can. 1089–1094.

EPSTEIN, E. 1972. Mineral nutrition of plants: principles and perspectives. John Wiley & Sons. New York, NY. 412 p.

EVANS, R. D., AND F. H. RIGLER. 1980. Measurement of whole lake sediment accumulation and phosphorus retention using lead-210 dating. Can. J. Fish. Aquat. Sci. 37: 817–822.

GODSHALK, G. L., AND R. G. WETZEL. 1976. Decomposition of macrophytes and the metabolism of organic matter in sediments. *In* H. L. Golterman [ed.] Interactions between sediments and freshwaters. Junk, The Hague. 473 p.

HESSLEIN, R. H. 1976. An in situ sampler for close interval pore water studies. Limnol. Oceanogr. 21: 912–914.

JONES, R. C. 1980. Primary production, biomass, nutrient limitation, and taxonomic composition of algal communities epiphytic on the submersed macrophyte *Myriophyllum spicatum* L. in a hardwater, eutrophic lake. Ph.D. thesis, University of Wisconsin-Madison, Madison, WI.

KIRKBY, E. A. 1969. Ion uptake and ionic balance in plants in relation to the form of nitrogen nutrition, p. 215–235. *In* I. H. Horison [ed.] Ecological aspects of the mineral nutrition of plants. Blackwell Scientific, Oxford.

KLUMP, J. V., AND C. S. MARTENS. 1981. Biogeochemical cycling in an organic rich coastal marine basin-II. Nutrient sediment – water exchange processes. Geochim. Cocmochim. Acta 45: 101–121.

LAZERTE, B. D. 1981. The relationship between dissolved carbon dioxide and its stable carbon isotope ratio in aquatic sediments. Geochim. Cosmochim. Acta 45: 647–656.

LEE, D. R. 1977. A device for measuring seepage flux in lakes and estuaries. Limnol. Oceanogr. 22: 140–147.

NICHOLS, D. S., AND D. R. KEENEY. 1976. Nitrogen nutrition of *Myriophyllum spicatum*: uptake and translocation of N by shoots and roots. Freshwater Biol. 6: 145–154.

SMITH, C. S. 1978. Phosphorus uptake by roots and shoots of *Myriophyllum spicatum* L. Ph.D. thesis, University of Wisconsin-Madison, Madison, WI.

SOMMERS, L. E., AND D. W. NELSON. 1972. Determination of total phosphorus in soils: a rapid perchloric acid digestion procedure. Soil. Sci. Am. Proc. 36: 902–904.

STAINTON, M. P., M. J. CAPEL, AND F. A. J. ARMSTRONG. 1977. The chemical analysis of fresh water. Miscellaneous Special Publication No. 25. Environment Canada. 120 p.

TESSENOW, U., AND Y. BAYNES. 1978. Redox-chemical effects of *Isoetes lacustris* L. in littoral sediment of Lake Feldsee (Black Forest). Arch. Hydrobiol. 82: 20–48.

WAISEL, Y., M. AGAMI, AND Z. SHAPIRA. 1982. Uptake and transport of ^{86}Rb, ^{32}P, ^{36}Cl and ^{22}Na by four submerged macrophytes. Aquat. Bot. 13: 179–186.

WETZEL, R. G. 1975. Limnology. Saunders, Philadelphia, PA. 743 p.

NOTES

Ultrasound Imaging Used to Detect Cysts of *Henneguya salminicola* (Protozoa: Myxozoa) in the Flesh of Whole Pacific Salmon

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Boyce, N. P. 1985. Ultrasound imaging used to detect cysts of *Henneguya salminicola* (Protozoa: Myxozoa) in the flesh of whole Pacific salmon. Can. J. Fish. Aquat. Sci. 42: 1312–1314.

Whole sockeye salmon, *Oncorhynchus nerka*, were scanned using an ultrasound imaging system. In real images, representing cross-sections of the soma, cysts (confirmed by subsequent dissection) were depicted as dark spheres against a lighter background of somatic muscle. This technique proved to be unsatisfactory with fish that were previously frozen.

Des saumons sockeye (*Oncorhynchus nerka*) entiers ont été examinés à l'aide d'un imageur à ultrasons. Dans les images réelles représentant des coupes transversales du soma, les kystes (révélés par la dissection ultérieure) apparaissaient comme des sphères foncées sur un fond clair de muscles somatiques. Cette technique ne s'est pas révélé satisfaisante pour des poissons auparavant congelés.

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The myxosporean parasite *Henneguya salminicola* (Protozoa: Myxozoa) forms spheroid or ovoid spore-filled cysts in the flesh of adult Pacific salmon (*Oncorhynchus* spp.). These cysts, up to 15 mm in diameter, are readily visible when the flesh is exposed, e.g. upon filleting, and their appearance detracts from the acceptability of the fish product. Domestic industry as well as foreign importers of whole salmon have expressed concern over the sporadic occurrence of high prevalences of infection in shipments of North American Pacific salmon. Since the presence of cysts is undetectable in whole fish, it has not been possible to institute appropriate strategies, e.g. culling at source, to alleviate the marketing problem. The need for a nondestructive technique for detecting cysts in whole fish has long been recognized, and the problem has been further exacerbated with the increasing export market for whole salmon in recent years.

A survey conducted on salmon spawning grounds in British Columbia in 1979 and 1980 (N. P. Boyce, unpubl. data) identified certain discrete stocks that were heavily infected with cysts of *H. salminicola*, which in turn provided a unique opportunity to conduct a range of studies on this parasite. Among the findings from these studies were that (1) all five British Columbia species of salmon are susceptible to infection and (2) the cysts occur largely in the posterior half of the fish (posterior to the leading edge of the dorsal fin).

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The survey also provided an adequate source of subject fish for testing the potential of ultrasound imaging for detection of *H. salminicola* cysts in the flesh of whole salmon.

Material and Methods

The fish used in the ultrasound trials were adult sockeye salmon, *Oncorhynchus nerka*, caught by seine in September 1980 at the entrance to Kennedy Lake, west coast of central Vancouver Island, British Columbia. At the time of capture these fish were still in marine water and in prime condition, essentially commercially marketable salmon. Prevalence of infection in this stock was determined by dissection and direct observation to be 20%, with infection intensities averaging 47 cysts per fish. Cyst diameter was 3–5 mm. Infected fish were selected for these trials; I used only fish in which cysts were revealed after a thin parasaggital section (approx. 4 cm in diameter) had been removed from the region of the caudal peduncle. Carcasses were held at 5°C and transferred within 28 h to the Department of Diagnostic Radiology, Vancouver General Hospital, where they were examined using the Picker Microview ultrasound scanning system (part No. 595701-1, Picker Corporation, 12 Clintonville Road, Northford, CT 06472).

A general account of the principles of ultrasonography is contained in Devey and Wells (1978). In very simple terms, the scanner head, mounted on an articulated arm, houses a 10-Mhz transducer which emits ultrasonic pulses that pass into the subject material. The transducer also receives echos returning

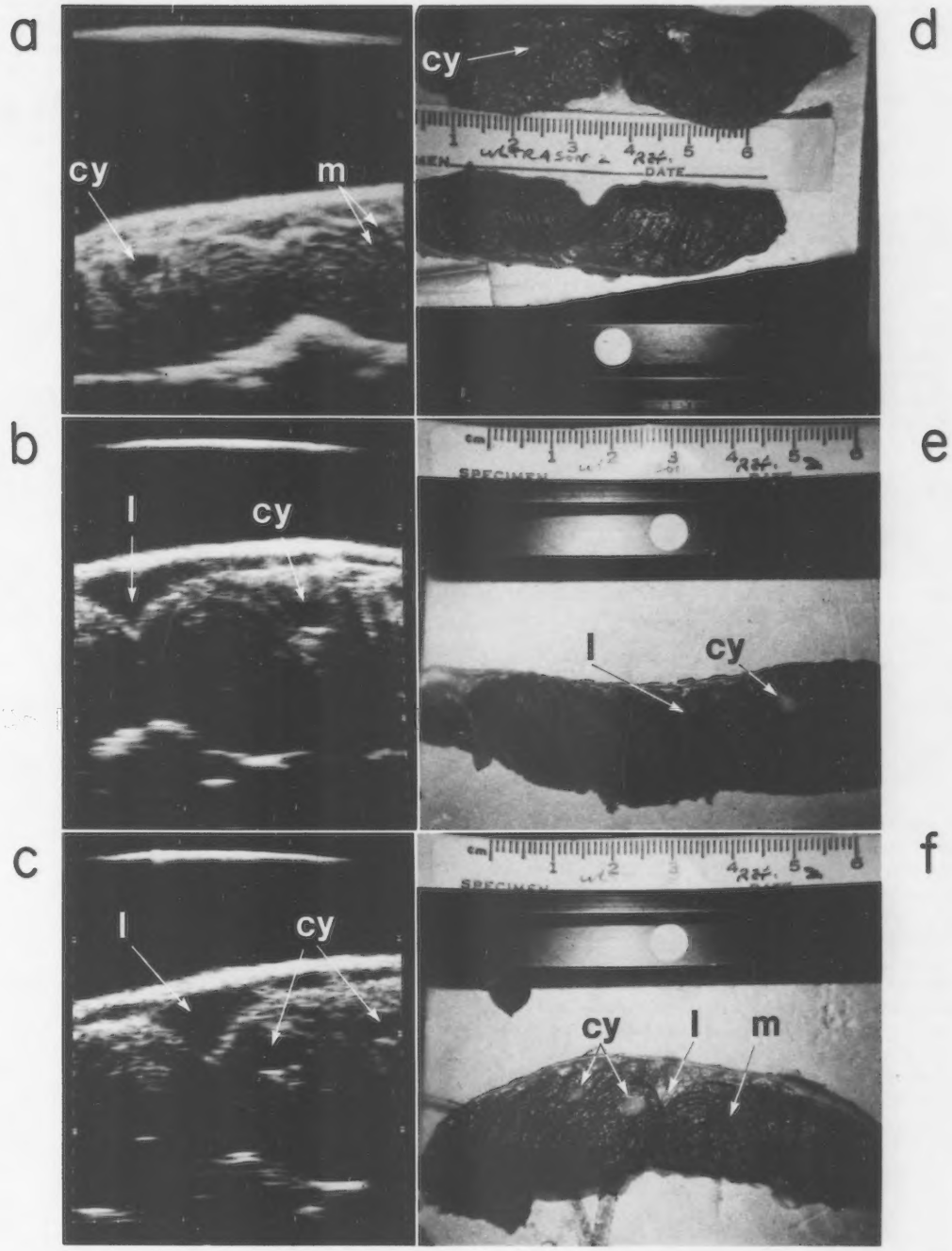

FIG 1. *Henneguya salmonicola* cysts in the flesh of sockeye salmon. (a–c) Ultrasound images of *Henneguya* cysts in body muscle; (d–f) light photographs of the same body muscle locations depicted in Fig. 1a–1c, showing *Henneguya* cysts. cy = cyst; m = myomere; l = M. lateralis superficialis.

from these pulses. The characteristics of each reflected echo signal (rate, intensity, etc.) vary with the degree to which the incident pulse was deflected, absorbed, reflected, scattered, etc. which in turn reflects the characteristics (shape, size, consistency, location, water content) of the tissue encountered by the ultrasonic pulse.

The transducer oscillates at 7½–30 sweeps per second, giving rise to a series of reflected signals which are integrated and

translated electronically into a real image displayed in rectangular format on a cathode ray tube. This image represents a vertical cross-section of the tissue, approximately 4 cm^2, lying beneath the scanner head. The scanning system includes a Polaroid camera for photography of the ultrasound-produced image.

With the scanner head held so that the transducer oscillates dorsoventrally over a salmon lying on its side, the image produced would be equivalent to a surface view of a salmon steak cut at the same location, approximately to the depth of the vertebral column (see Fig. 1d–1f). As the scanner head is directed along the length of the fish, the image on the screen is renewed continuously to reflect the internal features of the tissues beneath. The scanner head is held stationary for prolonged focussing on suspect material, or for ultrasound image photography.

Since ultrasound pulses are transmitted relatively poorly through the air, compared with water or tissue, an ultrasound-compatible gel smeared thickly on the fish was used initially to replace the air interface between the transducer and subject surface. Comparable results were obtained with the fish submerged in a basin of water and the transducer, also submerged, scanning at approximately 2–3 cm above the surface of the fish.

Results and Discussion

With the level of resolution and dynamic range provided by the 10-Mhz transducer, elements of the lateral musculature (myotomes, myosepta, M. lateralis superficialis) were depicted clearly, and *Henneguya* cysts were easily distinguishable, appearing as darker, roughly spherical inclusions against the relatively uniform background of lateral muscle.

Figure 1 shows ultrasound image photographs taken at various locations along the fish, and matching light photographs of transverse sections subsequently cut at the identical locations. The image reversal between paired Fig. 1c and 1f is due to difference in direction of focus (anteriad vs. posteriad).

While speed trials were not conducted, it is estimated that an experienced operator could effectively scan an entire adult sockeye salmon (both sides) in less than 1 min. Since, as indicated earlier, the cysts occur largely in the posterior portion of the fish, scanning may be restricted to that region of the body to further accelerate the examination process.

Repeated attempts to use ultrasound imaging to detect cysts in known infected fish that had been previously frozen and thawed were unsuccessful. According to Love (1966) and Bilinski et al. (1977), the freezing of animal tissue causes loss of intracellular tissue fluid which then accumulates in extracellular space. E. Bilinski (formerly at Department of Fisheries and Oceans, Technology Services Branch, Technology Research Laboratory, Vancouver, B.C. V6T 1X2, pers. comm.) suggested that these changes could alter the acoustic properties within our fish to the extent that the distinction between muscle and cyst becomes less detectable. Accordingly, previously frozen fish are not considered suitable subjects for this diagnostic method.

Freese (1969) demonstrated the application of an ultrasound technique to the detection of encapsulated helminths in the flesh of whole fish, viz. the cestode *Triaenophorus crassus* in whitefish (*Coregonus clupeaformis*) and the nematode *Porrocaecum* (now *Pseudoterranova*) *decipiens* in cod (*Gadus morhua*). The system described by Freese did not provide real images of the subject tissue and parasites, but produced instead a graphical wave display of the ultrasonic pulse echo structure which then required analysis and interpretation for distinguishing encapsulated helminths, and for estimating capsule size and location. Moreover, for adequate echo signal differentiation it was required that the encapsulated worms be rendered motile through the application of heat. The system described in the present paper provides a real image that requires minimal further interpretation.

There is little doubt that this system could be further modified for improved resolution and coverage. It is conceivable that a relatively simplified ultrasound imaging system could be designed specifically for fish examination.

The potential of ultrasound imaging for detecting other parasites or imperfections in the flesh of fish, e.g. internal bruises and tumors, remains to be explored. However, the range of capabilities already demonstrated for this technique in medical diagnostics suggests good prospects for its broad application in fisheries product quality inspection.

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References

BILINSKI, E., R. E. E. JONAS, Y. C. LAU, AND G. GIBBARD. 1977. Treatments before frozen storage affecting thaw drip formation in Pacific salmon. Can. J. Fish. Aquat. Sci. 34: 1431–1435.

DEVEY, G. B., AND P. N. T. WELLS. 1978. Ultrasound in medical diagnosis. Sci. Am. 238: 98–112.

FREESE, M. 1969. Ultrasonic inspection of parasitized whole fish. FAO Tech. Conference on Fish Inspection and Quality Control, Halifax, N.S. Paper FTC/69/0/IV. 13 p.

LOVE, M. 1966. The freezing of animal tissue, p. 317–405. *In* H. T. Meryman [ed.] Cryobiology. Academic Press, Inc., London and New York.

Standing Stock and Production of Fish in a Cascading Lake System on the Canadian Shield

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To examine factors influencing fish biomass and production, I carried out a multiple mark and recapture program in a cascading lake system on the Canadian Shield. Standing stock and production were determined for all "large" fish (vulnerable to the gear in use) in the system. Total fish biomass (range 5.2–24.8 $kg \cdot ha^{-1}$) decreased with progression downstream and with increasing depth. Production ranged from approximately 2.2 to 6.6 $kg \cdot ha^{-1} \cdot yr^{-1}$ and operated in a manner identical to standing stock. Salmonid flesh production, however, formed a greater proportion to overall fish production in the downstream, less productive lakes. Biomass was strongly influenced by lake depth in this watershed, and this was confirmed by examination of other published data for fish biomass and production in Shield lakes.

Dans le but d'étudier les facteurs influant sur la biomasse et la production piscicoles, l'auteur a mené un programme mixte de marquage et de recapture dans un système de lacs en cascade du Bouclier canadien. Le stock actuel et la production ont été déterminés pour tous les « gros » poissons vulnérables à l'engin utilisé dans le système. La biomasse piscicole totale (variant de 5,2 à 24,8 $kg \cdot ha^{-1}$) baissait vers l'aval et avec l'augmentation de la profondeur. La production a varié d'environ 2,2 à 6,6 $kg \cdot ha^{-1} \cdot an^{-1}$ de façon identique aux stocks actuels. La production de chair de salmonidés représentait toutefois une plus grande partie de la production piscicole globale dans les lacs d'aval moins productifs. La profondeur d'un lac influait fortement sur la biomasse dans ce bassin hydrographique; des données publiées sur la biomasse et la production piscicoles de lacs du Bouclier ont confirmé cette hypothèse.

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Fisheries managers have gained considerable predictive capability for estimating fish yield from empirical relationships among harvest data and lake abiotic variables (Ryder 1965; Ryder et al. 1974; Hanson and Leggett 1982; Schlesinger and McCombie 1983; Prepas 1983). However, yield data have been used as a readily available surrogate for standing stock and production, even though these latter traits are of particular importance when managers choose to partition yield or to evaluate stresses upon a fishery; notable exceptions are Rupp and DeRoche (1965), Chadwick (1976), and Fraser (1981). The latter studies originated in small lakes that were reclaimed by rotenone prior to stocking, suggesting that at least species composition was undesirable for that region.

Since the sparse published literature on biomass and production has forced the use of, at times, questionable yield data, I assessed abundance, biomass and production in a cascading lake system on the Canadian Shield supporting salmonids. Further, I examined the consistency of the relation between fish biomass and production and lake features in the available data.

Study Area

The Turkey Lakes Watershed is located on the Canadian Shield some 50 km north of Sault Ste. Marie, Ontario (approximately 47°N, 84°W). The overall basin is 10.5 km^2 and contains a chain of four oligotrophic lakes. The lakes vary from the small headwater Batchawana Lake (each basin about 6 ha) to the large deep (52 ha, 12 m) downstream Big Turkey Lake (Table 1).

The watershed is underlain by sparingly soluble silicate bedrock (greenstones and granite) and is overlain by thin, discontinuous glacial till. The lakes are chemically dilute, reflecting the parent lithology. Lakes range in average pH from 5.9 to 6.7.

Methods

Trap nets (2 m deep, 34 m leads, mesh size 3.81 cm stretched measure) were installed perpendicular to shore in each lake. Nets were tended daily during each of the sampling periods except on weekends. Effort was increased when success was low. Since the first year yielded no fish in trap nets set in the headwater lake (Batchawana), multifilament gill nets were set and lifted regularly for the remainder of 1979 and for 1980; since no fish were caught, effort was later concentrated on the downstream lakes.

When trap nets were lifted, all fish were removed from the net and transferred to a container of approximately 30–50 L of water. Each fish was measured, a scale sample removed (except burbot (*Lota lota*)), marked with a numbered spaghetti tag (Floy Tag and Mfg., Inc.) inserted between the interneural spines beneath the dorsal fin (Dell 1968), and released.

Population abundance estimates from the capture–mark–

TABLE 1. Size, mean and maximum depths, shoreline length, and grand means for selected chemical data collected from the five basins in the watershed. Data are in part from Jeffries and Semkin (1982) and in part from D. S. Jeffries (unpubl.).

	Lake area (ha)	Mean depth (m)	Max. depth (m)	Shoreline length (km)	Conductivity ($\mu S \cdot cm^{-1}$)	pH	Alkalinity ($\mu eq \cdot L^{-1}$)	SO_4 ($mg \cdot L^{-1}$)	Total P ($mg \cdot L^{-1}$)	Total N ($mg \cdot L^{-1}$)
Batchawana L.										
N. basin	5.9	3.9	11.3	1.31	23.4	5.95	55.5	5.51	0.0055	0.493
S. basin	5.8	3.3	10.9	1.61	27.0	6.06	71.7	6.10	0.0057	0.459
Wishart L.	19.2	2.2	4.5	3.14	33.3	6.53	132.1	6.23	0.0051	0.433
Little Turkey L.	19.2	6.0	13.0	2.15	38.8	6.57	167.8	6.40	0.0062	0.427
Big Turkey L.	52.0	12.2	37.0	5.91	41.1	6.65	185.3	6.38	0.0042	0.360

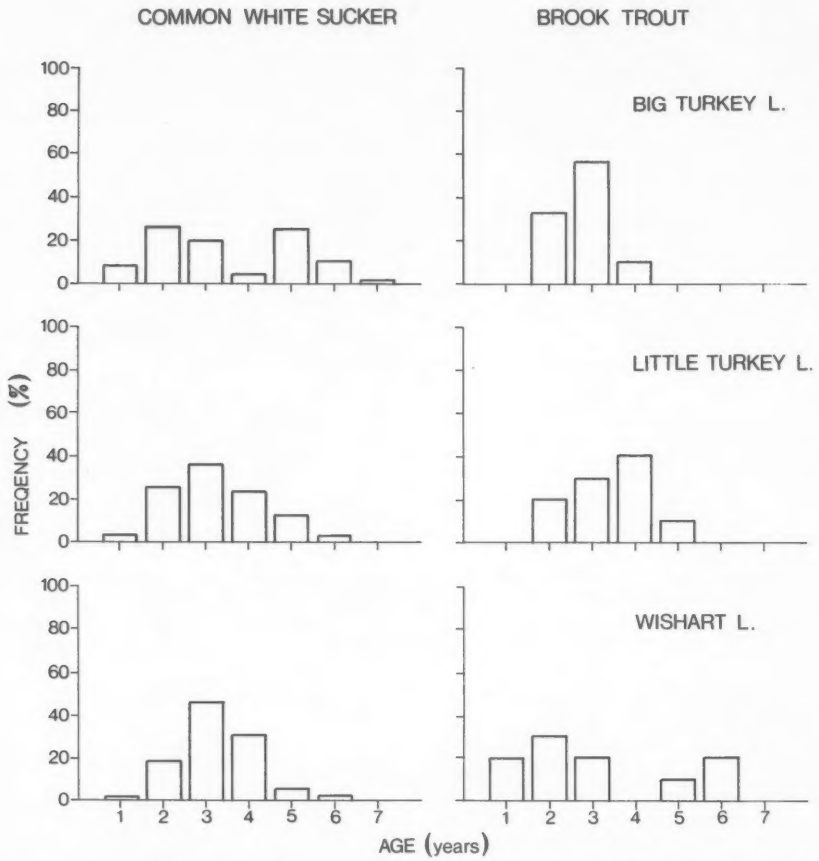

FIG. 1. Frequency distributions by age for white sucker and brook trout from the three lakes in the watershed, all years of collection. Number of aged fish is 987 for white sucker and 98 for brook trout.

recapture data were calculated by the Petersen technique for white sucker (*Catostomus commersoni*) and the Schumacher–Eschmeyer method for brook trout (*Salvelinus fontinalis*) (see Ricker 1958).

To reduce handling stress, weights were taken from only a small sample of fish from each of the three lakes. Data were pooled and length–weight regressions developed for white sucker ($\log_e w = -5.79 + 2.02 \log_e L$, $r = 0.89$, $N = 84$) and brook trout ($\log_e w = -11.75 + 3.09 \log_e L$, $r = 0.98$, $N = 52$). Mean lengths at age for each population estimate were converted to weight using the developed regressions. The instantaneous growth in weight (g) was then calculated for the period between each population estimate.

Since sample sizes were at times small for certain ages, estimates of number were not made by age group. Rather, the estimate of total number (N) was partitioned according to the age structure of the sample. Total biomass was then determined by adding the estimates of weight for each age group.

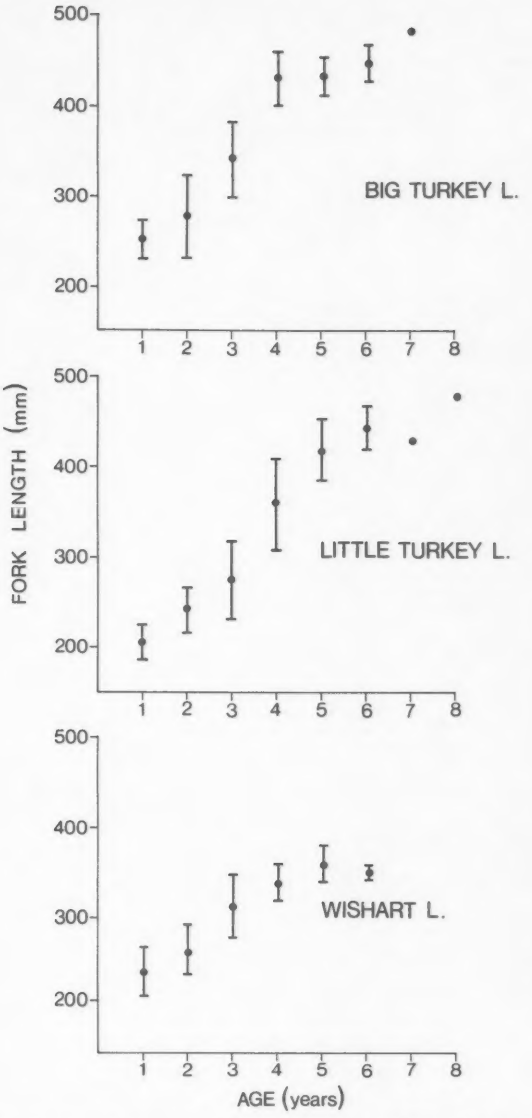

FIG. 2. Length at age for white sucker from the three lakes in the watershed, all years combined.

Average biomass of the stock ($\bar{B}$), defined simply as the stock vulnerable to capture, was calculated as the mean of two adjacent biomass estimates (Chapman 1968). Production was then calculated using $P = g\bar{B}$.

Results

No fish were caught in Batchawana Lake. Trap nets installed for the mark–recapture program were in place for 25–62% of the ice-free period representing a substantial expenditure of effort in each lake.

Five fish species are common to the three lakes including brook trout, white sucker, burbot, creek chub (*Semotilus atromaculatus*), and brook stickleback (*Culaea inconstans*). Both Little Turkey and Big Turkey contain lake trout (*Salvelinus namaycush*). Three other cyprinids, emerald shiner (*Notropis atherinoides*), lake chub (*Couesius plumbeus*), and northern redbelly dace (*Phoxinus eos*), are common to the two Turkey lakes.

Considerable differences in the age-class structure of two of the common species, white sucker and brook trout, exist (Fig. 1). Missing or weak year-classes occurred in the white sucker population of Big Turkey Lake and the brook trout population of Wishart Lake. White sucker appear to be fully vulnerable at age 3 and brook trout at age 2 (Fig. 1). No white sucker were older than 7 yr, and the most abundant age groups were 2, 3, or 4. The age-class structure of brook trout differed significantly among the lakes. Fish from Big Turkey and Little Turkey lakes were similar in size but white sucker from Wishart Lake were approximately 15% shorter by age 5. Growth of white sucker in Wishart Lake appeared to slow after age 3 (Fig. 2).

Although population estimates were made for white sucker in the spring and fall of 1980 and 1981 and in the spring of 1982, only those periods when the sample was greater than 20% of N were used (Robson and Regier 1964), thus reducing bias in the estimate (Table 2). White sucker were more abundant in Wishart Lake where there were almost three times the number that existed in the other two lakes. Survival approached 50% for the vulnerable population in Wishart and Big Turkey lakes but was greater, approximately 75%, in Little Turkey Lake.

Since the sample size for brook trout was small for the spring and fall netting periods, I used all of the data to calculate a single estimate of abundance (Table 3) using the Schumacher–Eschmeyer method. Overall, brook trout abundance increased with progression downstream in the watershed. The number of brook trout in Big Turkey Lake was approximately three times that in Wishart Lake. Confidence limits of the estimate of N were narrow in each case.

Problems in estimating abundance of other resident fishes in the watershed were encountered. Although lake trout exist in Little Turkey Lake, they are rare, as only one was captured during 3 yr of netting effort. In Big Turkey Lake, nine lake trout were marked in the fall of 1980 and all were captured by anglers. This happened again when eight fish were marked in the fall of 1981.

Tagged burbot did not readily intermingle with the unmarked population. Fish were recaptured only at the release site and approximately 85% were recaptured during the period of marking.

Biomass estimates of white sucker averaged 425.6, 166.9, and 211.5 kg in Wishart, Little Turkey, and Big Turkey lakes, respectively; annual production decreased uniformly downstream (Table 4). The ratio of production to biomass was highest in Little Turkey Lake and lowest in Big Turkey Lake.

For brook trout (Table 5), biomass was highest (58.6 kg) in Big Turkey Lake and similar (approximately 42 kg) in Wishart and Little Turkey lakes. Production followed the same pattern.

Estimates of total biomass and production from my data were as follows:

	Wishart	Little Turkey	Big Turkey
Biomass ($kg \cdot ha^{-1}$)	24.8	10.9	5.2
Production ($kg \cdot ha^{-1} \cdot yr^{-1}$)	6.6	3.9	1.2

Small fish, cyprinids, and burbot do contribute to total biomass; however, from the literature (Rupp and Deroche 1965; Chadwick 1976; Fraser 1981), omission of the "small" fish not

TABLE 2. Estimates of abundance (N) and survival (S) for white sucker from the three lakes in the watershed for periods when capture sample is 20% of N. Survival rates were adapted from Ricker's (1958) equation 5.2. Standard deviations (±) of the estimate are in parentheses.

	Batchawana L.		Wishart L.		Little Turkey L.		Big Turkey L.	
	N	*S*	*N*	*S*	*N*	*S*	*N*	*S*
Spring 1980	0		886 (154)		636 (176)		483 (314)	
								0.45 (0.14)
Fall 1980			1488 (845)			0.75 (0.34)	358 (82)	
				0.46 (0.19)				0.38 (0.34)
Spring 1981			1481 (641)		392 (93)		533 (307)	
								0.36 (0.21)
Fall 1981							396 (171)	
								0.57 (0.48)
Spring 1982								

TABLE 3. Estimates of abundance for other species in the watershed. Confidence limits (95%) are in parentheses.

	Batchawana L.	Wishart L.	Little Turkey L.	Big Turkey L.
Brook trout	0	82 (12–184)	114 (69–198)	287 (159–338)
Lake trout	0	0	1 capture	Exploitation high
Burbot	0	Population recapture rate 1 in 2	Segregation 1 in 4	1 in 3

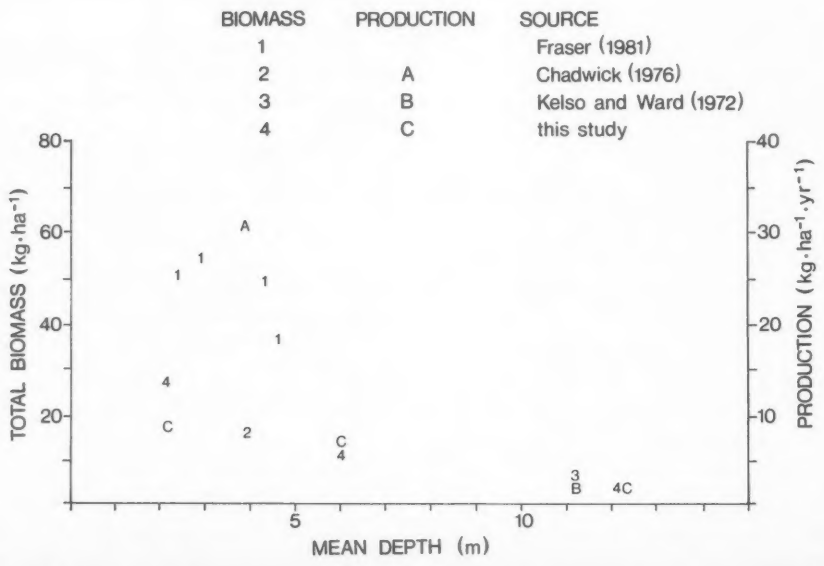

FIG. 3. Relation between biomass and production and lake depth from data available in eastern North America.

TABLE 4. Estimates of biomass (B, kg) and production (P, kg·yr^{-1}) for white sucker in three lakes in the watershed.

Age	$\bar{L}$	$\bar{W}$	g	B_1	B_2	B_3	B_4	$\bar{B}$	P_1	P_2	P_3	P_4	$\bar{P}$	P/B
White sucker, Wishart L., all years														
1	239	195	0.1993		9.285			3.095						
2	264	238	0.3684	2.720	169.989	24.673		65.794	0.271	17.865	2.459		6.865	
3	317	344	0.1126	121.914	220.105	244.543		195.521	22.958	71.855	49.590		48.134	
4	335	385	0.1403	151.794	10.312	245.179		135.762	15.410	12.973	27.571		18.651	
5	359	443	0	52.987	5.866	11.809		23.554	14.365	1.135	18.028		11.176	
6	347	414		0.220		5.457		1.892						
Total				329.635	415.557	531.661		425.618	53.004	103.828	97.648		84.826	0.20
White sucker, Little Turkey L., all data														
1	205	143	0.3254	0.855	1.794			1.325						
2	241	198	0.2686	45.082	21.266			33.174	7.474	3.752			5.613	
3	275	259	0.5546	56.006	36.042			46.024	13.576	7.697			10.636	
4	362	451	0.3004	40.730	34.298			37.514	26.825	19.505			23.165	
5	420	609	0.1205	43.768	15.517			29.643	12.692	7.482			10.087	
6	446	687		16.603	12.927			14.765	3.637	1.714			2.676	
7	429	635			3.983			1.915		≈0				
8	479	794			4.980			2.490		≈0				
Total				203.044	130.807			166.850	64.204	40.150			52.177	0.31
White sucker, Big Turkey L., all data														
1	254	220	0.1899		18.509	5.863	2.091	6.616						
2	279	266	0.4302		40.662	70.889	20.541	33.023		5.618	7.288	2.150	3.764	
3	345	409	0.4416		19.328	16.350	86.975	30.663		12.904	18.765	23.127	13.699	
4	430	638	0.0278	18.489	13.476	34.005	43.203	27.293		7.292	11.194	28.939	11.856	
5	436	656	0.0505	199.614	34.523	61.188	18.964	78.527	3.032	0.667	1.323	0.864	1.471	
6	447	690	0.1689	99.981		36.777		34.189	7.565		2.474		2.510	
7	486	817		4.735				1.184	8.843				2.211	
Total				322.819	126.498	225.072	171.774	211.540	19.440	26.481	41.044	55.080	35.511	0.17

TABLE 5. Estimates of biomass (B, kg) and production (P, kg·yr^{-1}) for brook trout in three lakes in the watershed.

Age	$\bar{L}$	$\bar{W}$	g	B	P	P/B
Brook trout, Wishart L.						
1	181	75	0.4700	1.230	6.751	
2	211	120	0.6197	1.968	2.309	
3	258	223		5.485		
4			1.1818		6.763	
5	378	727	0.8141	5.961	13.381	
6	492	1641		26.912		
Total				41.556	23.204	0.56
Brook trout, Little Turkey L.						
1						
2	214	125	0.7208	2.850	4.195	
3	270	257	0.5147	8.789	7.308	
4	319	430	0.7530	19.608	11.301	
5	407	913		10.408		
Total				41.655	22.804	0.55
Brook trout, Big Turkey L.						
1	150	42	1.3561	0.723	10.957	
2	233	163	0.3044	15.437	7.369	
3	257	221	0.5072	32.982	10.768	
4	303	367		9.480		
Total				58.622	29.094	0.49

studied in this experiment would likely only increase biomass estimates by 7–18%.

From aerial creel census surveys on 46 lakes in this region (Ontario Ministry of Natural Resources, unpubl. data), I determined that there was no significant difference ($P < 0.05$) in fish harvest during winter and summer. Therefore, I multiplied the total estimated time fished (57.5 h in Little Turkey Lake and 310 h in Big Turkey Lake) by the average yield of 0.13 fish·angler^{-1}·h^{-1} (Armstrong and Bernier 1982) to determine that anglers caught 8 fish from Little Turkey Lake and 41 fish from Big Turkey Lake. This harvest represents a yield of 0.6 kg·ha^{-1}·yr^{-1} from Little Turkey Lake and 1.0 kg·ha^{-1}·yr^{-1} from Big Turkey Lake.

Although biomass and production decreased with progression downstream, the contribution by salmonids to the "total" biomass increased downstream (20% in Wishart Lake, 29% in Little Turkey Lake, and 45% in Big Turkey Lake).

Discussion

Although estimates were made for only some of the species in the watershed, it is clear that standing stocks and production of fishes decreased with progression downstream, except salmonids whose contribution increased downstream. In contrast, Fraser (1981) found that small Shield lakes exhibited community conservatism; however, his lakes were dominated by white sucker. In these lakes, although the salmonid – white sucker community persisted, varying lake features resulted in signifi-

cant differences in standing stock and production and differences in species proportion in the community.

Primary production as measured by ^{14}C assimilation has also been measured in this watershed (Collins et al. 1983), and unit production tended to decrease with increasing depth. That is, production was greatest in Wishart Lake and lowest in Big Turkey Lake, exactly following depth (Table 1).

To better understand the factors controlling standing stock and production, I used published results of biomass, production, and lake features (Rupp and DeRoche 1965; Kelso and Ward 1972; Chadwick 1976; Fraser 1981) for oligotrophic lakes in this climatic zone. From these data (Fig. 3), as mean depth increased, fish standing stock and production decreased. The regression between biomass and mean depth (biomass = $48.2 - 3.8\bar{z}$, $r = 0.72$, $N = 8$) indicated that a relation exists with biomass influenced strongly by changes in depth. On the other hand, there was no significant correlation between alkalinity (related to TDS, C. K. Minns, Department of Fisheries and Oceans, Burlington, Ont., unpubl.) and biomass. These data are supported by Prepas (1983), who also found that depth was as good a predictor of fish production (using yield data) as TDS or its correlates. Other chemical features, including nutrient levels, varied little in the watershed. Unfortunately, data for production are even fewer but the five lakes with available production figures (from Kelso and Ward 1972; Chadwick 1976; this study) followed the pattern observed for biomass.

Available turnover rates (P/B) suggest that lower ratios should exist for higher trophic levels (Emlen 1973). Ratios were low for both brook trout and white sucker (range 0.17–0.56) by comparison with the cyprinids and percids of Red Deer Lake (range 1.96–2.70). Interestingly, the P/B ratio for brook trout in Red Deer Lake was approximately three times that observed in this watershed, likely reflecting the more stable, self-reproducing stock existing in this watershed.

It is evident that lake features strongly influence stock and generated production. It is also likely that $\bar{z}$ alone may be a sufficiently strong predictor of biomass (and probably production). These and published data indicate that, among the lake features measured, lake morphometry exerts the major influence upon stock and production, similar to factors influencing fish yield (Prepas 1983).

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References

ARMSTRONG, D., AND M-F. BERNIER. 1982. Sault Ste. Marie district aerial fishing pressure survey winter and summer 1981. Ont. Minst. Nat. Res. Intern. Rep. 11 pages plus tables and appendices. (Available from District Office, Sault Ste. Marie, Ont.)

CHADWICK, E. M. P. 1976. Ecological fish production in a small Precambrian Shield lake. Environ. Biol. Fishes 1: 13–60.

CHAPMAN, D. W. 1968. Production. *In* S. D. Gerking [ed.] Methods for assessment of fish production in fresh waters. Int. Biol. Prog. Handb. 3: 313 p.

COLLINS, R. H., J. R. M. KELSO, J. H. LIPSIT, AND J. E. MOORE. 1983. Phytoplankton production, as estimated by the C^{14} technique, and populations contributing to production 1980/81/82, in the Turkey Lakes, Ontario, watershed. Can. Tech. Rep. Fish. Aquat. Sci. 1191: 23 p.

DELL, M. B. 1968. A new fish tag and rapid cartridge fed applicator. Trans. Am. Fish. Soc. 97: 57–59.

EMLEN, J. M. 1973. Ecology: an evolutionary approach. Addison Wesley Publ. Co., Don Mills, Ont. 493 p.

FRASER, J. M. 1981. Estimates of the standing stocks of fishes in four small Precambrian Shield Lakes. Can. Field-Nat. 95: 137–143.

HANSON, J. M., AND W. C. LEGGETT. 1982. Empirical prediction of fish biomass and yield. Can. J. Fish. Aquat. Sci. 39: 257–263.

JEFFRIES, D. S., AND R. SEMKIN. 1982. Basin description and information pertinent to mass balance studies of the Turkey Lakes watershed. Turkey Lakes Watershed Rep. No. TLW-82-01: 34 p.

KELSO, J. R. M., AND F. J. WARD. 1972. Vital statistics, biomass, and seasonal production of an unexploited walleye (*Stizostedion vitreum*) population in West Blue Lake, Manitoba. J. Fish. Res. Board Can. 29: 1043–1052.

PREPAS, E. E. 1983. Total dissolved solids as a predictor of lake biomass and productivity. Can. J. Fish. Aquat. Sci. 40: 92–95.

RICKER, W. E. 1958. Handbook of computations for biological statistics of fish populations. Bull. Fish. Res. Board Can. 119: 300 p.

ROBSON, D. S., AND H. A. REGIER. 1964. Sample size in Petersen mark–recapture experiments. Trans. Am. Fish. Soc. 93: 215–226.

RUPP, R. S., AND S. E. DEROCHE. 1965. Standing crops of fishes in three small lakes compared with C^{14} estimates of net primary productivity. Trans. Am. Fish. Soc. 94: 9–25.

RYDER, R. A. 1965. A method for estimating the potential fish production of north-temperate lakes. Trans. Am. Fish. Soc. 94: 214–218.

RYDER, R. A., S. R. KERR, K. H. LOFTUS, AND H. S. REGIER. 1974. The morphoedaphic index, a fish yield estimator — review and evaluation. J. Fish. Res. Board Can.31: 663–688.

SCHLESINGER, D. A., AND A. M. MCCOMBIE. 1983. An evaluation of climatic, morphoedaphic and effort data as predictors of yields from Ontario sport fisheries. Ont. Fish. Tech. Rep. Ser. No. 10: iii and 14 p.

Adenovirus-Like Particles Associated with a Disease of Cultured White Sturgeon, *Acipenser transmontanus*

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Hedrick, R. P., J. Speas, M. L. Kent, and T. McDowell. 1985. Adenovirus-like particles associated with a disease of cultured white sturgeon, *Acipenser transmontanus*. Can. J. Fish. Aquat. Sci. 42: 1321–1325.

Juvenile white sturgeon, *Acipenser transmontanus*, suffering from a chronic mortality had enlarged nuclei particularly in the epithelium of the straight intestine and spiral valve. These nuclei were often five times larger than those of uninfected cells and contained numerous virus particles with an average diameter of 74 nm. Although nuclear changes induced by the virus are similar to those described for certain members of the Herpesviridae, the virion morphology and absence of an envelope were, however, more characteristic of the Adenoviridae. Attempts to isolate the virus using established cell lines from selected freshwater fish and two lines recently developed from white sturgeon were unsuccessful. Nuclear enlargement indicating virus infection was, however, observed in juvenile sturgeon receiving intraperitoneal injections of homogenates of viscera from infected fish.

Des noyaux dilatés, surtout dans l'épithélium de l'intestin droit et de la valvule spiralée, étaient présents chez des esturgeons blancs (*Acipenser transmontanus*) juvéniles frappés de mortalité chronique. Ces noyaux, qui étaient souvent cinq fois plus gros que ceux des cellules non infectées, contenaient de nombreuses particules virales d'un diamètre moyen de 74 nm. Quoique les modifications nucléaires provoquées par le virus soient semblables à celles qui sont décrites pour certains membres de la famille Herpesviridae, la morphologie du virion et l'absence d'une enveloppe étaient plus caractéristiques de la famille Adenoviridae. On n'a pas réussi à isoler le virus à l'aide de lignées cellulaires établies de poissons dulçaquicoles choisis et de deux lignées de l'esturgeon blanc récemment développées. La dilatation des noyaux révélatrice d'une infection virale a par contre été observée chez des esturgeons juvéniles recevant des injections intrapéritonéales d'homogénats de viscères de poissons infectés.

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The potential of utilizing captured white sturgeon, *Acipenser transmontanus*, to obtain seed stocks for aquaculture facilities has recently been evaluated (Dorshov et al. 1983). This species grows rapidly, and under usual circumstances is quite hardy when cultured. Furthermore, juveniles produced by culture can be used to replenish natural stocks depleted by overfishing and pollution.

After three successful years of rearing sturgeon obtained from wild sexually mature adults, severe mortalities were recorded by many of the growers obtaining stocks from the Sacramento River (California) in 1984. Much of the mortality was attributed to recurrent infections with gill and body myxobacteria and was accompanied by signs of starvation and lethargy prior to death. However, during the course of an examination of fish from one private farm with similar signs but no evidence of bacterial infection, a new viral disease was observed. We report here on the characteristics of the disease associated with a new virus that infects cultured white sturgeon.

Materials and Methods

Fish — The diseased juvenile white sturgeon (0.5 g avg. wt.) were obtained from a local farm and were the progeny of brood stock taken from the Sacramento River. The fish were maintained in 4-ft circular fiberglass tanks at an average water temperature of 19°C. The fish had been fed a diet consisting of brine shrimp (*Artemia* sp.) and dry trout feed before the time diseased animals were examined. Juvenile sturgeon (10.0 g avg. wt.) from another stock, with no signs of disease, were used in experiments designed to determine whether the virus could be transmitted by intraperitoneal injections of tissues from smaller diseased sturgeon (see transmission trials).

Light and electron microscopy — Fish were fixed in Davidson's solution for 24–48 h and then transferred to 70% ethanol containing 3% HCl. After embedding in paraffin, tissue was sectioned at 5–7 μm and stained with hematoxylin and eosin. Sections of the spiral valve were placed for 1 h in Karnovsky's fixative and then transferred to phosphate-buffered saline (0.01 mol/L, pH 6.8). These specimens were then postfixed in 1% OsO_4, embedded in Epon plastic, and then sectioned (<100 nm) and stained with lead citrate and uranyl acetate. Examinations and photography were done on a Philips EM 400 transmission electron microscope.

Virus isolation and transmission trials — Attempts to isolate the virus from infected tissues were made according to McDaniel (1979). The following cell lines from freshwater fish were

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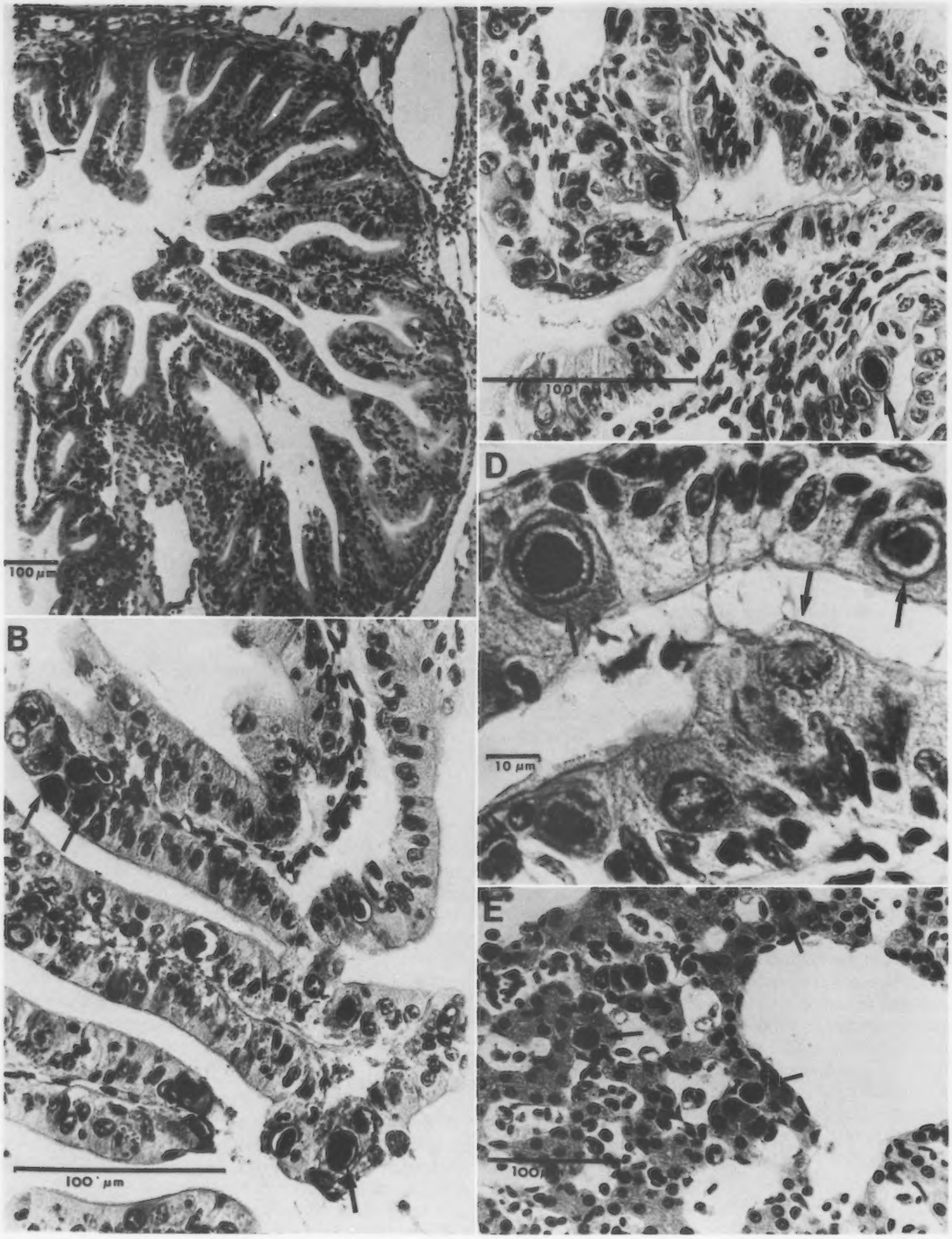

FIG. 1. Light micrographs of histological sections stained with hematoxylin and eosin made from white sturgeon, *Acipenser transmontanus*, infected with an adeno-like virus. (A) Low magnification of straight intestine showing numerous enlarged nuclei in the epithelium. (B) Higher magnification of straight intestine. (C) Low magnification of the epithelium of the spiral valve showing enlarged nuclei. (D) Higher magnification of several nuclei in different stages of enlargement in the spiral valve. (E) Similar nuclear changes in the liver. Arrows indicated enlarged nuclei.

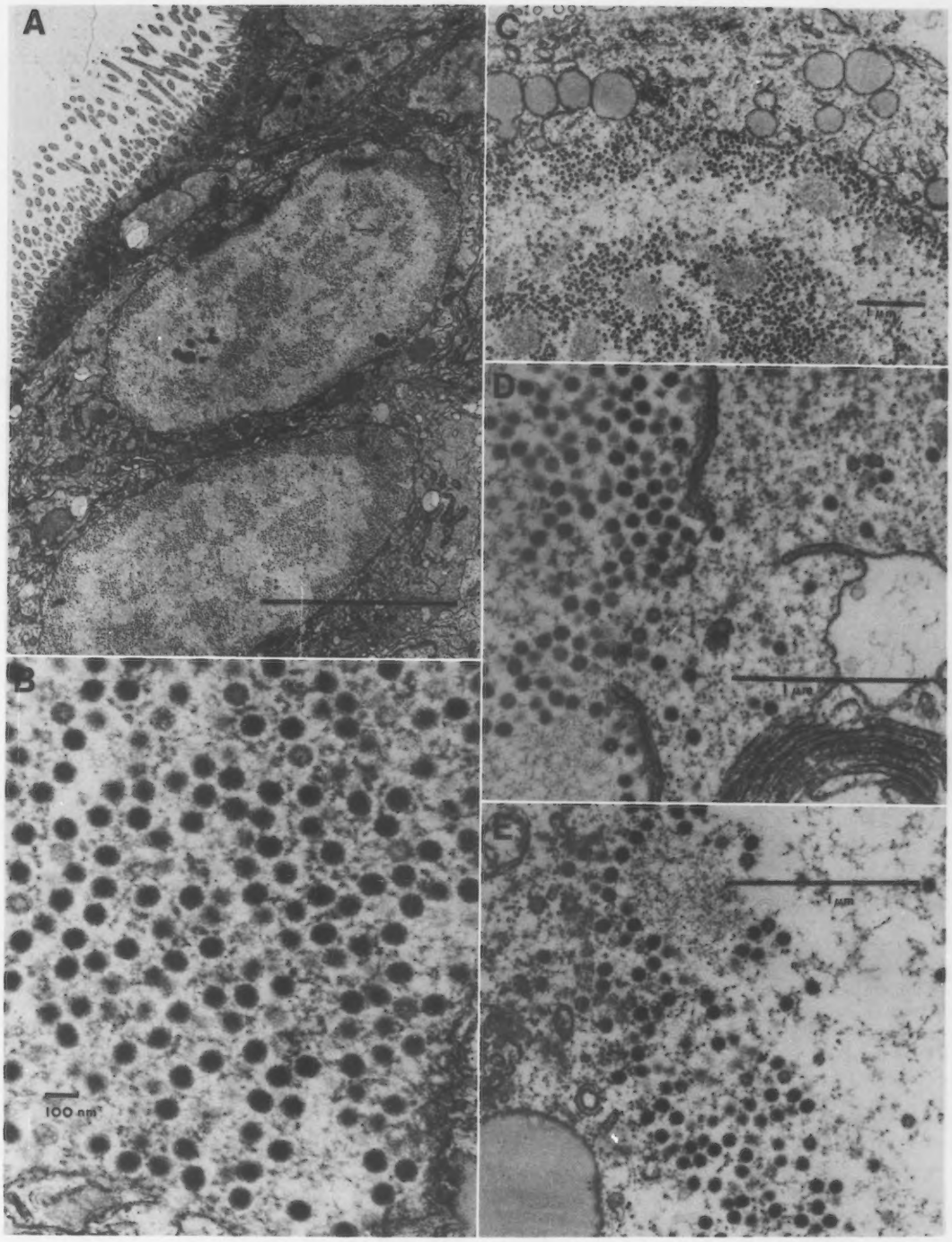

FIG. 2. Electron micrographs of the adeno-like virus of white sturgeon, *Acipenser transmontanus*. (A) Epithelium lining the spiral valve showing normal and greatly enlarged nuclei. Cilia on the epithelial cell surface are also evident. (B) Higher magnification of virions in enlarged nuclei showing double capsids present in many of the virions. (C) Enlarged nuclei showing nuclear membrane disintegration and apparent release of virions. (D) Released particles in cell cytoplasm via a break in the nuclear membrane and (E) near complete disintegration of the nuclear membrane.

inoculated with suspensions made from infected sturgeon viscera: BF-2 from blue gill (*Lepomonis macrochirus*), BB from brown bullhead (*Ictalurus nebulosus*), FHM from fathead minnow (*Pimephales promelas*), CCO from channel catfish (*Ictalurus punctatus*), and CHSE-214 from chinook salmon (*Oncorhynchus tshawytscha*). In addition, two recently established cell lines from white sturgeon heart (SH-1) and spleen (SS-2) tissues (unpublished data) were similarly inoculated. All lines were grown at 25°C except the CHSE-214 line at 20°C in minimal essential medium (MEM) supplemented with 5% fetal calf serum, 2 mmol of glutamine, 50 μg/mL streptomycin, and 50 I.U./mL penicillin. Following inoculation with suspensions made from infected sturgeon, all lines were incubated with the same media at 20°C and observed daily. In addition, eight juvenile sturgeon (10.0 g avg. wt.) from a population with no clinical signs of disease were inoculated intraperitoneally with homogenates made from the viscera of infected fish. Each fish received 0.1 mL of filtered (0.45 μm) homogenate (1:10, w/v) and were then placed in tanks with 16°C flowing water. Control fish receiving inoculations with MEM (0.1 mL) held under the same conditions (but in separate tanks) were examined along with experimental sturgeon. Four weeks after treatment the fish were sacrificed and examined for histological changes indicating nuclear enlargement and presumptive virus infections.

Results

Signs of infection — Affected sturgeon were lethargic, anorexic, and emaciated. Internally, the liver was pale and the gut devoid of food but no other remarkable signs were evident.

The most prominent histological feature of tissues from infected fish was the nuclear hypertrophy of the epithelial cells lining the straight intestine and spiral valve (Fig. 1A–1D). In certain fish, up to one third of the cells of the epithelia were involved. Nuclei of affected cells (up to five times the diameter of normal cells) showed an apparent progression of development of increased enlargement and eventual eruption into the lumen of the gut. Identical nuclear changes were also observed in the liver parenchyma of one diseased fish (Fig. 1E).

Electron microscopy — An examination of affected tissues from the spiral valve and straight intestine by electron microscopy showed that nuclei contained large numbers of electron-dense hexagonal virions (Fig. 2A). An average diameter calculated from the measurement of 50 virions was 74 nm. Several electron-lucent stages were seen and prominent inner and outer capsids with diameters of 47 and 74 nm, respectively, were observed (Fig. 2B). Virions were almost always contained within the nuclear membrane. The particles were apparently released either as a result of breaks in (Fig. 2D) or disintegration of (Fig. 2E) the nuclear envelope. There were no indications of virions budding through membranes even in areas where large numbers of particles were adjacent to the nuclear envelope (Fig. 2D).

Virus isolations and transmission trials — All attempts to cultivate the virus in established cell lines were unsuccessful and cytopathic effects (CPE) were never observed. Co-cultivation of trypsinized cells from affected tissues with the sturgeon cell lines SS-2 and SH-1 was similarly unsuccessful in propagating the virus. Primary cultures made from affected tissues are still being monitored but to date have failed to show CPE or release of virus even after blind passage of the culture fluids onto the SH-1 and SS-2 cell lines.

Juvenile sturgeon receiving intraperitoneal injections of visceral homogenates from infected fish showed nuclear enlargement in the gut epithelium identical to that observed in naturally infected fish, while all control fish were free of such changes. The numbers of inclusions, however, in experimental infections were considerably less than those observed in naturally infected fish.

Discussion

Recent reviews by Wolf and Mann (1980) and Wolf (1984) list viruses either observed or isolated from fish but presently do not include any representatives from sturgeon. Our report describes what we believe to be the first observation of a virus in this species. The agent was associated with a disease characterized by chronic mortality of anorexic and emaciated fish. Fish with these signs showed frequent nuclear changes in the straight intestine and spiral valve (Fig. 1A–1E). However, even apparently healthy fish in the same tank showed nuclear changes but at a substantially reduced incidence. The gut epithelium seemed to be the target issue although occasionally a more systemic condition existed. We presume that a heavy involvement of the gut epithelium would substantially alter the fish's ability to absorb nutrients, eventually causing the emaciation and death observed in cultured sturgeon.

The virus causes prominent changes in the nucleus, and abundant particles are obvious upon examination by electron microscopy (Fig. 2A). The virions appear to be double-shelled. The nuclear changes are similar to those induced by herpesviruses of the genus *Cytomegalovirus* (Rapp 1983). Several herpesviruses have been isolated from fish and their characteristics have been described (Wolf and Darlington 1971; Wolf et al. 1975; Kimura et al. 1981; Kelley et al. 1983). These viruses, however, tend to induce cytological changes characterized by syncitia formation rather than the nuclear hypertrophy observed in sturgeon.

A herpesvirus of cultured turbot (*Scophthalmus maximus*) described by Buchanan and Madeley (1978) and Richards and Buchanan (1978) and a herpes-like virus of Pacific turtles (Frye et al. 1977) induce nuclear inclusions that are similar to those we have observed. However, the morphology and size of the virions associated with the condition in turbot and turtles are clearly similar to previously described representatives of the family Herpesviridae. In contrast, the diameter of virions in our study averaged 74 nm compared with 100 nm expected for other herpesviruses, and enveloped particles were never observed. The size and shape of the virions in our study more closely resembled the adenovirus-like particle described by Jensen and Bloch (1980) in Baltic cod (*Gadus morhua*) with an unknown skin disease. The icosahedral virus they described was unenveloped and 77 nm in diameter and showed an electron-lucent outer capsid surrounding a more dense inner "nucleolus." However, in contrast with the virus from sturgeon, Jensen and Bloch (1980) observed fibers (20–25 nm) emanating from the vortex pentons of the virion, a feature characteristic of adenoviruses but difficult to visualize by electron microscopy unless negative staining is used (Palmer and Martin 1982). In addition, the remarkable nuclear changes induced by the sturgeon virus were not mentioned as features of the cod adenovirus.

Methyl green pyronin stains of infected cells in the sturgeon epithelium showed that the nuclear inclusions contained DNA, and since grouping of the DNA viruses is dependent on size, morphology, and presence of an external envelope, we must be

cautious with our grouping of the virus from sturgeon. Although certain characteristics are consistent with the family Adenoviridae, the absence of fibers or filaments projecting from the vertex pentons and distinct inner and outer capsids makes placement in this group tentative. Further biochemical characterization of the virus, either extracted directly from infected tissues or from cell cultures, will be needed for proper taxonomic placement.

Studies now in progress are aimed at confirming the infectious nature of the disease and its effects on the successful culture of white sturgeon. Several attempts at transmitting the agent by intraperitoneal inoculation of healthy sturgeon showed that the virus can be passed by cell-free filtrates of tissues from diseased fish. However, signs of the disease as originally described were not noted and the incidence of nuclear inclusions was very low.

Although not an explosive condition, the disease associated with this new virus in at least one farm has caused a chronic condition that eventually resulted in a cumulative mortality of 50% over a 4-mo period. This indicates that under certain conditions the virus may hinder the successful culture of white sturgeon.

Acknowledgements

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References

BUCHANAN, J. S., AND C. R. MADELEY. 1978. Studies on *Herpesvirus scophthalmi* infection of turbot *Scophthalmus maximus* (L.): ultrastructural observations. J. Fish. Dis. 1: 283–295.

DORSHOV, S. I., W. H. CLARK JR., P. B. LUTES, R. L. SWALLOW, K. E. BEER, A. B. MCGUIRE, AND M. D. COCHRAN. 1983. Artificial propagation of white sturgeon, *Acipenser transmontanus*, Richardson. Aquaculture. 32: 93–104.

FRYE, F. L., L. S. OSHIRO, F. R. DUTRA, AND J. D. CARNEY. 1977. Herpesvirus-like infection in two Pacific pond turtles. J. Am. Vet. Med. Assoc. 171: 882–884.

JENSEN, N. J., AND B. BLOCH. 1980. Adenovirus-like particles associated with epidermal hyperplasia in Cod (*Gadus morhua*). Nord. Veterinaermed. 32: 173–175.

KELLY, R. K., O. NIELSEN, S. C. MITCHELL, AND T. YAMAMOTO. 1983. Characterization of *Herpesvirus vitreum* isolated from hyperplastic epidermal tissue of walleye, *Stizostedion vitreum vitreum* (Mitchill). J. Fish Dis. 6: 249–260.

KIMURA, T., M. YOSHIMIZU, M. TANAKA, AND H. SANNAOHE. 1981. Studies on a new virus (OMV) from *Oncorhynchus masou* I. Characteristics and pathogenicity. Fish Pathol. 15: 143–147.

MCDANIEL, D. 1979. Procedures for the detection and identification of certain fish pathogens. American Fisheries Society, Fish Health Section, Bethesda, MD. 112 p.

PALMER, E. L., AND M. L. MARTIN. 1982. An atlas of mammalian viruses, p. 103–108. CRC Press, Inc., Boca Raton, FL.

RAPP, F. 1983. The biology of Cytomegaloviruses, p. 1–66. *In* B. Roizman [ed.] The herpesviruses. Plenum Press, New York, NY.

RICHARDS, R. H., AND J. S. BUCHANAN. 1978. Studies on *Herpesvirus scophthalmi* infection of turbot *Scophthalmus maximus* (L.): histopathological observations. J. Fish Dis. 1: 251–258.

WOLF, K. 1984. Fish viruses: their biology, classification, hosts, pathology and control, p. 197–215. *In* E. Kurstak and R. G. Marusyk [ed.] Control of virus diseases. Marcel Dekker Inc., New York, NY.

WOLF, K., AND R. W. DARLINGTON. 1971. Channel catfish virus: a new herpesvirus of Ictalurid fish. J. Virol. 8: 523–533.

WOLF, K., AND J. A. MANN. 1980. Poikilotherm vertebrate cell lines and viruses: a current listing for fishes. In Vitro 16: 168–179.

WOLF, K., T. SANO, AND T. KIMURA. 1975. Herpes-virus disease of salmonids. Fish Disease Leaflet 44. U.S. Dep. Interior, Fish and Wildlife Service Division of Cultural Methods Research, Washington, DC 20240.

LETTERS AND COMMENTS/LETTRES ET COMMENTAIRES

Correction in Use of Oxycalorific Equivalent

In a recent communication from Dr. C. H. Boggs (Southwest Fisheries Center, La Jolla, CA) I was advised of a significant error in the calculation of the locomotor energetics of adult sockeye salmon (Brett 1973). The objective of that research was to compare the estimates of energy expended when determined by measuring the oxygen consumption of swimming, starving salmon with that derived from the caloric loss in body substance. Oxygen consumption rates can be transformed into calories by use of the oxycalorific equivalent, which at that time was taken from mammalian physiology, namely, 4.86 cal/mL O_2, or 3.40 cal/mg O_2. Experiments were conducted on fingerling and adult salmon. The opportunity to perform synchronous measurements of metabolic rate and body energy-loss was afforded for fingerlings only, in a tunnel respirometer; for the adults the average metabolic rate was derived from measuring the water velocity in an exercising flume and translating this into equivalent swimming energetics by use of the nomograms in Brett and Glass (1973). The differences for the adults, as reported, were underestimated.

There were four possibilities for explaining the difference in the separately derived estimates of energy expenditure, in which the assumed oxygen consumptions were significantly less than the measured body loss, namely, (1) unrepaid anaerobic metabolism, (2) unrecorded or low estimates of oxygen consumption, (3) inappropriate oxycalorific equivalent for fish, and (4) compounding of error estimates. It was suggested by C. H. Boggs that anaerobic metabolism might account for much of the difference, a possibility inherent in the work of Krueger et al. (1968).

My comments and choice of explanation were dealt with in my response to Dr. Boggs in which I wrote:

> The major error that I see which occurred in the calculation of the adult sockeye salmon was that the metabolic value in *mg* O_2 was mistakenly used as *mL* O_2, i.e., the energy expended should have been multiplied by 0.7, resulting in 243 kcal (Group 1) and 393 kcal (Group 2). This would alter the text table on p. 1805 to read 0.517 kcal/kg/hr (Group 1) and 0.712 kcal/kg/hr (Group 2). The energy difference between estimated O_2-consumed and determined body-loss would then be +54% (Group 1) and +29% (Group 2).
>
> Had I determined these correctly, particularly Group 2 which appeared to support the proposition completely, I would have reviewed in the paper where the possibilities of difference could occur. Turning to the nomograms in Brett and Glass (1973), these are of course best minimum approximations; they provide an estimate (±10%) of the oxygen consumption *solely* for locomotion at the imposed velocity. No account of excitability or other activity demands on metabolism (including ovary development) are involved. Under Materials and Methods, on p. 1800 (Brett 1973), there is an introductory paragraph for adult sockeye noting outbursts of aggression. The significance of this is illustrated in Fig. 2 for fingerlings in the respirometer with elevated metabolic rates. Recently, while working with fully mature pink salmon in a tunnel respirometer, we have recorded elevated metabolic rates which we suspect are influenced by hormonal release. The nomograms in Brett and Glass (1973) involved very few fully mature fish and none in the late migratory stage. Consequently, I do not find it difficult to presume that the *unmeasured* actual O_2-consumption of the exercised adults was considerably higher than the *deduced* minimum locomotor demand. At the time, because of believing the derived differences to be no more than about 20%, I did not deem it necessary to dig further. Krueger et al. (1968) was dealing with differences of over 200%, and also using the derived locomotor O_2-demand without any recognition of the excitement factor.

I would like to take this opportunity to comment further on the possible explanations as noted:

Anaerobic metabolism

It is certainly possible that salmon might store or excrete anaerobic by-products of metabolism. The most likely of these is lactate, which in fatigued salmon reaches intolerable levels in muscle and blood. However, the exercised salmon operated at sustained speeds at about one half their maximum, covering equivalent distances of 563 and 933 km. When fingerlings were tested in the respirometer at the termination of one experiment, they showed a normal ability for burst speeds.

Cumulative anaerobic metabolism in reptiles is well documented, but does not occur during their fairly sluggish, sustained locomotion (Bennett 1978).

Low estimates of oxygen consumption

When measuring metabolism in a tunnel respirometer a fish has to perform at the imposed velocity; when swimming in front of a shocking device it can do more but not less. Repeated hourly measures of metabolic rate show a wide variation that is greatest at low velocities and least at high velocities approaching the active metabolic rate. This phenomenon was reported in Brett (1964, p. 1191, fig. 4) and noted again in Brett (1965). It represents excess energy expended by bursts (anaerobic), fluttering, oxygen-debt repayment, and general excitability. Consequently, when determining the locomotor cost of swimming, a line is drawn through those points showing least oxygen consumption at any given sustained velocity (Brett 1964, p. 1192, fig. 5). Hence, the line is interpretive, and not subject to regression analysis. Error is estimated from a set of such lines. This phenomenon has been overlooked in more than one case when referring to sockeye energetics. Included herewith is a plot of the corrected energy costs (Fig. 1) determined for Group 1 (n = 4 adult females) and Group 2 (n = 8), and the

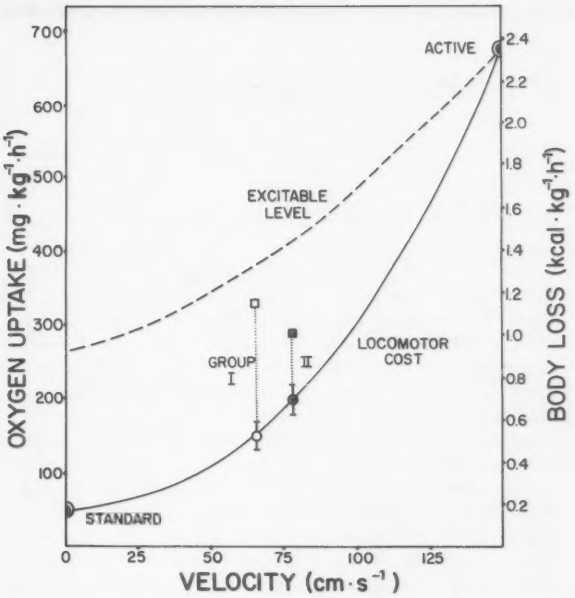

FIG. 1. Energy expenditure of two groups of adult sockeye salmon swimming in a flume at the velocities indicated. Squares represent the estimated body caloric loss (corrected); circles (±2 SE) represent the expected oxygen uptake rate attributed to locomotion only, using an approximated curve from Brett and Glass (1973). The upper broken line depicts the general level of metabolic rate that frequently occurs from spontaneous activity and excitability in captivity.

minimum locomotor costs (±2 SE) derived from Brett and Glass (1973). The general, upper level of "excitement" metabolic rate is included.

Oxycalorific equivalent for fish

Because fish excrete mainly ammonia rather than urea, as in mammals, and derive a lot of their sustained energy from protein sources, Brafield and Solomon (1972) determined a more appropriate equivalent, namely, 3.25 cal/mg O_2. This only differs from the mammalian equivalent by about 4%.

Compounding error

The initial energy content of the fish that are forced to swim has to be estimated by sampling other fish of similar size and stage, at zero time. A small error (say 10%) becomes progressively larger as the caloric content of the exercised fish falls, e.g. 20% error for half expended. Losses of tissue (epithelial, endothelial, erythrocyte destruction, etc.) have some effect on final dry body weight. Unrecorded bursts of activity (as noted) may register as final caloric loss without equivalence in oxygen consumption.

Conclusion

Without detailed measurements including sampling for excreted anaerobic by-products, and sufficient numbers and controls to provide sensitive estimates of confidence limits, the resolution of the recorded difference between the two measures of energy expenditures remains unexplained. Salmon are not only jumpers but migrators, capable of maintaining continuous swimming over hundreds of miles without rest. The inefficiencies of anaerobic metabolism without aerobic repayment appear to me to be too great for evolution to have overlooked the wily salmon — J. R. Brett, *Pacific Biological Station, Nanaimo, B.C. V9R 5K6.* (J8158)

References

BENNETT, A. F. 1978. Activity metabolism of the lower vertebrates. Annu. Rev. Physiol. 40: 447–469.

BRAFIELD, A. E., AND D. J. SOLOMON. 1972. Oxycalorific coefficients for animals respiring nitrogenous substrates. Comp. Biochem. Physiol. 443: 837–841.

BRETT, J. R. 1964. The respiratory metabolism and swimming performance of young sockeye salmon. J. Fish. Res. Board Can. 21: 1183–1226.

1965. The swimming energetics of salmon. Sci. Am. 213: 80–85.

1973. Energy expenditure of sockeye salmon, *Oncorhynchus nerka*, during sustained performance. J. Fish. Res. Board Can. 30: 1799–1809.

BRETT, J. R., AND N. R. GLASS. 1973. Metabolic rates and critical swimming speeds of sockeye salmon, *Oncorhynchus nerka*, in relation to size and temperature. J. Fish. Res. Board Can. 30: 379–387.

KRUEGER, H. M., J. B. SADDLER, G. A. CHAPMAN, I. J. TINSLEY, AND R. R. LOWRY. 1968. Bioenergetics, exercise, and fatty acids of fish. Am. Zool. 8: 119–129.

BOOK REVIEWS/COMPTES RENDUS

Engineering Approaches for Lake Management, Volume 1: Data Analysis and Empirical Modeling; Volume 2: Mechanistic Modeling. *Kenneth H. Reckhow and Stephen C. Chapra. Butterworth Publishers (Ann Arbor Science), Boston, London. 1983. 832 p. $85 (U.S.), 2-volume set (combined).*

The title of this two-volume set is somewhat misleading. The books deal almost exclusively with a single aspect of lake management, that is, with trophic status or degree of nutrient enrichment. If one is familiar with the authors' backgrounds and published papers, this emphasis is not at all surprising; as long as readers (or buyers) realize this, they will not be disappointed. There is, in fact, a chapter on toxic substances, and nonnutrient examples are used (infrequently) in illustrating the other 16 chapters, but, by and large, the books are about phosphorus.

Volume 1 contains three sections. The first, which is on scientific inference, includes an interesting but rather cursory discussion of the scientific method, a very useful presentation of some basic definitions that nonmodellers in particular will find valuable, and, most significantly, a treatment of uncertainty that clearly and concisely sets the stage for much of what follows in the two volumes. After reading the first section, one is left with a clear picture of the scientific philosophy of the authors, a fact that is very satisfying and one that occurs on reading very few text books.

Section 2 covers data analysis and includes a chapter on basic statistical concepts. The material is covered in most elementary textbooks and could have been substantially reduced here; nevertheless, it does provide a good introduction to the subsequent discussions. A chapter on robust statistics is more useful, whereas a final chapter on sampling design will be particularly helpful because the subject is discussed with trophic status problems specifically in mind. Although the subject has been covered many times, the treatment here is particularly good.

Section 3, which is titled "Empirical Modeling," is the core of the first volume. The theory of regression analysis and linear models is followed by a discussion of trophic status indices, and, finally, by a very detailed chapter on the use of empirical models for lake trophic state evaluation. The latter is unquestionably the focal point of the volume. It is well written, includes good useful examples that make the presentation easy to follow, and very nicely synthesizes the preceding chapters including the use of uncertainty analysis. Although it is a minor point, I would describe much of this section as semi-empirical rather than empirical; that is, some of the models are simply empirical modifications of an approach that has a mechanistic basis, the mass balance approach.

I am a little less enthusiastic about Volume 2. After a short introduction, input–output models are discussed in detail. There is an unclear and I think artificial line drawn between the "empirical" mass balance models of Volume 1 and the mechanistic mass balance models of Volume 2. I would have preferred to see the two chapters integrated, or at least divided on a different basis. The treatment of the input–output models in Volume 2 is both quite sophisticated mathematically and very well done. Even the more complex models are presented in such a way that they will be accessible to the average reader.

The next section contains detailed discussion of spatial resolution in modelling: one chapter describes horizontal and one describes vertical resolution. Again, these chapters are very good syntheses of complex problems, and will be of special interest to those interested in large lakes such as the Laurentian Great Lakes. The sixth section discusses models of kinetic resolution, beginning with nutrient – food chain models. However, unlike most of the preceding material, this chapter will have more limited usefulness. The models are complex enough that data collection (e.g. for model confirmation) becomes difficult. A brief section on inorganic carbon, pH, and acidity in the middle of this chapter seems somewhat out of place. Once again, however, the authors maintain their consistent approach by rigorously applying uncertainty analysis to these very complex food chain models. I would have been happy if the authors had stopped here. Instead, the second part of this section discusses toxic substance models, and provides the first real departure from trophic status considerations. Although the application of the principles exposed earlier to other substances is commendable, a more thorough treatment than can be given in a single chapter would be preferable. The final section, which is on model implementation, provides a fair summary and synthesis of the preceding chapters.

The major contribution of these books lies in the rigorous application of proper data analysis techniques and statistical methods to trophic status management. Many readers will recognize that this has been an objective of at least one of the authors for the past decade. Another major contribution is that these books make some of the more complex aspects of lake modelling palatable to those without a knowledge of advanced mathematics. The progression from empirical to simple mechanistic (or semi-empirical) to spatial to kinetic models is generally matched by an increase in model complexity and an increase in resistance to utilize the models by all but the specialists. These books will serve a useful purpose if they can help break down some of this resistance. The frequent use of specific numerical examples throughout also helps take the mystique out of the equations.

The production of the book is very good. I found few typographical errors, and although I certainly did not check each of the more than 900 equations, I saw no mistakes in any of those that I paid close attention to.

Dr. Richard Vollenweider notes in the foreword to these books that they are not for the systems analyst but rather for "students, research workers, engineers and managers of more practical orientation." I agree fully; for these people the books are essential.

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BOOKS RECEIVED/LIVRES REÇUS

Business management in fisheries and aquaculture. *Ian Chaston. Fishing News Books Ltd., Farnham, Surrey, England. 1984. 136 p. £7.50. ISBN 0-85238-132-8.* The objective of this book is to present some of the important issues in modern management theory and to show how these can be applied to fisheries and aquaculture. Selected issues within the functional skills of finance, marketing, production, and personnel management are covered, as well as the concept of strategic management. [R648]

Introduction to the practice of fishery science. *William F. Royce. Academic Press Inc., Orlando, FL. 1984. 428 p. $35.00 (U.S.), £27.50 (U.K.) ISBN 0-12-600960-0.* The three parts of this book contain (a) a treatment of the profession itself, (b) an update of the author's 1972 book on the traditional fishery sciences, and (c) a presentation of an overall qualitative concept of the activity of fishery scientists. The approach is, insofar as possible, worldwide and treats social as well as technical problems. The emphasis is on principles and issues rather than details of technique or scientific problems. [R649]

Proceedings of the North American oyster workshop. Special Publication No. 1. *Kenneth K. Chew (editor). Louisiana Statement University, Baton Rouge, LA. 1982. 300 p.* This is the first special workshop sponsored by the World Mariculture Society. It was held 6–8 March 1981 in Seattle, WA, and was primarily an industry workshop to address issues affecting the oyster industry. Areas of present and future problems are expressed and solutions are suggested. [R650]

Ecotoxicology. Ecological Bulletins 36. *Lennart Rasmussen (editor). Publishing House of the Swedish Research Councils, Stockholm, Sweden. 1984. 170 p. ISSN 0346-6868, ISBN 91-86344-18-8.* Proceedings of the Third Oikas Conference held 30 November – 2 December 1982 in Copenhagen, Denmark. Main topics covered were environmental behavior of pollutants; ecotoxicological effects on animals, plants, plankton, microorganisms, and on ecosystems; and the use of ecotoxicological principles in administration and environmental protection. [R651]

Fundamentals of aquatic toxicology: methods and applications. *Gary Rand and Sam R. Petrocelli (editors). Hemisphere Publishing Corporation, New York, NY. 1985. 666 p. $69.95 (U.S. and Canada) (cloth), ISBN 0-89116-302-5; $39.95 (U.S. and Canada) (paper), ISBN 0-89116-382-4. Outside the United States, distributed by the McGraw-Hill International Book Company.* Over 30 contributors present descriptions of basic concepts and test methods of aquatic–chemical interactions along with data and interpretations. The five parts are as follows: toxicity testing, sublethal effects, specific chemical effects, chemical distribution/fate, and hazard evaluation. The book serves as a text for students as well as a reference. [R652]

A phytogeographical study of South Greenland. Vascular plants. Meddelelser Om Gronland, Bioscience 15. *Jon Feilberg. 1984. ISBN 87-17-05229-7.* [R653]

Fishes of the North-Eastern Atlantic and the Mediterranean, Vol. I. *P. J. P. Whitehead, M.-L. Bauchot, J.-C. Hureau, J. Nielsen, and E. Tortonese (editors). Unesco Publications No. 56, Paris. 510 p. fr250. ISBN 92-3-002215-2.* The first of a three-volume reference work by more than 70 specialists consisting of a complete faunistic survey (1256 species from 218 families) of the sea fishes of the Atlantic northeast and Mediterranean with keys, diagnoses, line drawings, and biological data. Volume 1 treats 64 families — Petromyzonidae to Anotopteridae. The major aim of this contribution is to assure the correct identification of species and to summarize what is known of their habitat preferences, food, reproduction, and geographical distribution. [R654]

Marine birds: their feeding ecology and commercial fisheries relationships. *David N. Nettleship, Gerald A. Sanger, and Paul F. Springer (editors). Special publication, Canadian Wildlife Service. Available free on a first come first served basis from Distribution Section, Canadian Wildlife Service, Department of the Environment, Ottawa, Ont., Canada K1A 0E7. 1984. 220 p. ISBN 0-662-13311-0.* Proceedings of the Pacific Seabird Group Symposium, Seattle, WA, 6–8 January 1982. This symposium consisted of three parts as follows: the feeding ecology of marine waterfowl, the feeding ecology of pelagic marine birds, and seabird and commercial fisheries interactions. Attention was focussed on the immense flocks of waterfowl that play an important role in marine ecosystems, particularly in the Northeast Pacific. [R655]

Fish population dynamics in tropical waters: a manual for use with programmable calculators. ICLARM Studies and Reviews 8. *Daniel Pauly. International Center for Living Aquatic Resources Management, Metro Manila, Philippines. 325 p. $14.00 (U.S.) surface, $17.50 (U.S.) air mail (paper); $25.00 (U.S.) surface, $29.50 (U.S.) airmail (cloth). ISSN 0115-4389, ISBN 971-1022-03-6 (cloth), ISBN 971-1022-04-4 (paper).* This book presents a variety of methods for stock assessment, all of which have been tested for their applicability to tropical fish. Emphasis is given to length–frequency methods. The full program listings and user instructions of 30 programs for use with HP 67/97 programmable calculator are included. The objective of this contribution is to give clearly and simply instructions on how to analyze data collected so that fish stock assessment is much easier for its practitioners. [R656]

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